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*(Recognosce notum, ignotum inspice)*

# TRANSACTIONS

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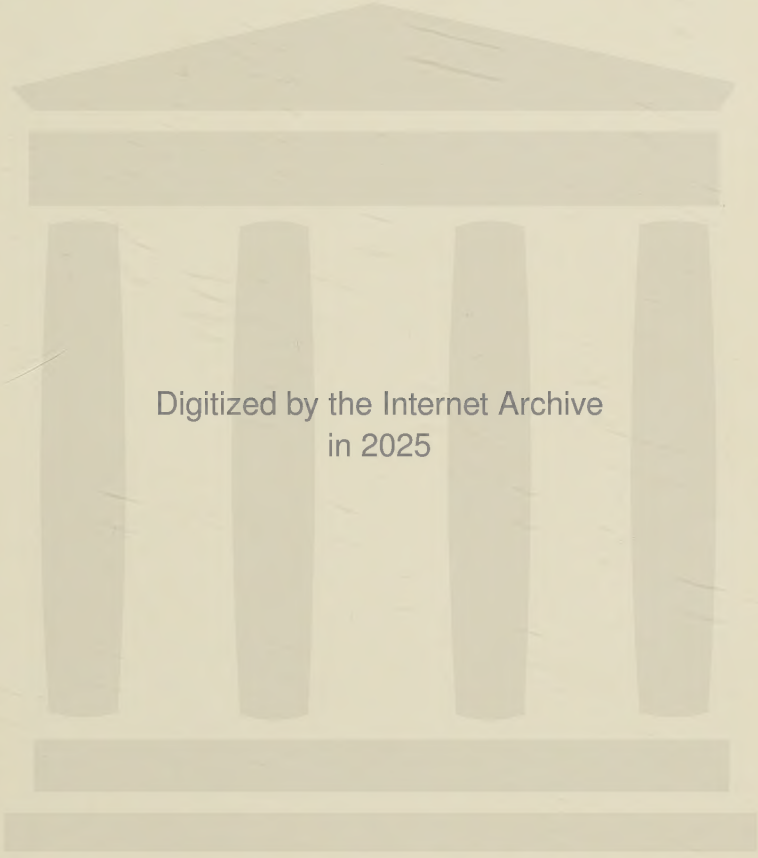
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Dr WATSON

(*Facing p.* 581)

*Trans. Brit. mycol. Soc.* **43** (4), 581–582 (1960).

# OBITUARY

## DR WALTER WATSON
1872–1960

Dr Walter Watson, schoolmaster, bryologist, lichenologist of international repute, and Honorary Member of this Society, who died at his home in Taunton on 10 January 1960 at the age of 87 had an uneventful but influential life. He was born on 30 March 1872 at Sicklinghall, Wetherby, Yorkshire, and after graduating from the Royal College of Science taught successively at Larne Grammar School (1901–2), Poulton-le-Fylde Grammar School (1902), and at Sexey's School, Bruton (1903–7), where he was botany master. In 1908 he joined the staff of Taunton School where he became senior science master and remained until his retirement in 1934. He was an able teacher and the present senior science master at Taunton School, Dr Ernest Neal (an authority on badgers), was one of his pupils.

Dr Watson was a man of remarkable energy. He was the author of *Readable School Botany* and *Elementary Botany*, and his many scientific papers included important contributions to a knowledge of lichens, on which he became a world authority.

In 1918 the first of a series of papers in the *Journal of Ecology* on the ecology of bryophytes and lichens of this country was devoted to the bryophytes and lichens of calcareous soil (part of a thesis for which he was awarded the D.Sc. by the University of London), and subsequent contributions dealt with those of fresh water (1919), arctic-alpine vegetation (1925), moorland (1932), beechwoods (1936), and other woodland types (1936). Among other major papers mention may be made of his helpful key for the determination of lichens in the field (*J. Bot., Lond.*, **60**, Suppl., pp. 28, 1922), the review of lichen classification (*New Phytologist Reprint*, No. 18, 1929), the list of British fungi parasitic on lichens (these *Transactions*, **31**, 305–339, 1948) and the monograph on *Usnea* in Great Britain and Ireland (*ibid.* **34**, 368–375, 1951).

He finally summarized the literature on the occurrence of lichens in the British Isles, in the light of a life-time's field work, in the *Census Catalogue of British Lichens*, which was published by the British Mycological Society in 1953. Correcting the proof sheets of this volume was Dr Watson's last scientific work. In addition, he made many contributions to local lists of lichens including the lichen lists for several of the Society's forays (these *Transactions*, **9**, 133–135, 1924 (Bristol); **21**, 12–15, 1937 (Totnes)). His lists of Somerset bryophytes, algae, mycetozoa, and lichens were published in the *Proceedings of the Somerset Archaeological and Natural History Society*. For many years he was a member of the Council of that Society and was President and Recorder of the Botanical Section. Dr Watson also served terms as president of the Yorkshire Naturalists Union and the Bryological Society, and in 1950 he was honoured as a vice-president of

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the VII International Botanical Congress at Stockholm. He was elected an Associate *honoris causa* of the Linnean Society in 1918. Always a keen cricketer, Dr Watson was at the time of his death the oldest member of the Somerset County Cricket Club.

Dr Watson was ever ready to give generously of his special knowledge and he had a wide circle of correspondents for whom he identified lichens from many parts of the world. He did much to interest others in the study of lichens in Britain. He gave his lichen herbarium to the Royal Botanic Gardens, Kew, and the remainder of his collections to the Somerset County Museum, Taunton.

I am grateful to Mr A. J. Dodd and Mr W. C. Moore for help in the preparation of this notice.

G. C. AINSWORTH

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# FUNGAL COLONIZATION OF STERILE HAIR IN CONTACT WITH SOIL

By D. M. GRIFFIN

*The School of Agriculture, University of Sydney, N.S.W., Australia*

(With 3 Text-figures)

The fungal colonization of sterile hair in contact with various soils was studied. A characteristic succession was demonstrated, the initial colonizers being common soil fungi of high general competitive saprophytic ability, the final ones slow-growing keratinolytic fungi. The succession varied in detail with different soils and these differences could in part be attributed to known soil factors. A comparison of the fungal flora of the soils as revealed by the technique here described and by Warcup's soil-plate method shows that the latter indicates a far greater preponderance of heavily sporing fungi with high competitive saprophytic ability than does the former, in which each colony on the given soil substrate yields but one isolate. The bearing of these experimental results on various concepts in soil ecology is discussed.

In recent years, many authors have discussed the problems and reviewed the progress associated with an understanding of the ecology of fungi in the soil. There would seem to be general agreement that three of the major (and inter-related) problems are concerned with the difficulty of differentiating between dormant and active portions of the fungal population which can be identified in the soil at any given time; with measuring, in some sense, the activity of fungi on natural soil substrates; and with ascertaining the precise pattern of activity of the fungi on these substrates. Recently many new techniques and results have been published concerned with the first two problems, of which those of Brown (1958), Grossbard (1958), Harley & Waid (1955), Parkinson (1957), Sewell (1956), Thornton (1958), Waid & Woodman (1957) and Warcup (1955*a*, *b*, 1957) should be noted. However, comparatively little progress has been made in attacking the third problem and Garrett (1955), speaking of present-day techniques, has said that their limitations 'can best be appreciated by supposing that an ecologist studying the vegetation of a meadow had to confine his observations to samples of herbage taken from a haystack'.

In an endeavour to obtain more specific information on these activity patterns, workers have studied the colonization of various substrates (DeVay, 1956), such as roots (Stenton, 1958), but there is usually great difficulty in interpretation due to the complexity of the substrate; an exception is the work of Tribe (1957) using Cellophane as a relatively pure cellulose source. Indeed the size and complexity of most natural substrates which are easily manipulated combined with the small size of the microenvironments inhabited by the individual fungal colonies go a long way towards explaining the difficulty of investigating the substrate patterns within the soil, for as Stanier (1953) has stated: 'A single cellulose fibre

provides a specialized environment with its own characteristic microflora, yet may occupy a volume of not more than a cubic millimetre', and he goes on to explain the well-nigh universal geographical distribution of micro-organisms by the omnipresence of these specific micro-environments.

The investigations now to be described were designed to study the pattern of colonization and succession in the micro-environment presented by sterile human hair in contact with various soils. Although hair has been widely used as a bait for keratinolytic fungi (Karling, 1946, 1947, 1948; Vanbreuseghem, 1952*a*, *b*; Ajello, 1953, 1959; Gordon, 1953; Daniels, 1954; Durie & Frey, 1955, 1957; Frey & Durie, 1956; Sparrow, 1957; Stockdale, 1958; Dawson & Gentles, 1959), no detailed study of its colonization would appear to have been made. That hair is colonized by many fungi that are not keratinolytic is not surprising in view of the work of Bolliger (1951) and Bolliger & Gross (1952) on aqueous extracts from hair, which include uric acid, urea, ammonia, pentose, glycogen, phenols, amino acids, etc., in appreciable quantities. The peculiar advantages of hair for an investigation of the type here to be described will be detailed in the body of this paper. No study of the biochemical and physiological bases of the succession during colonization has yet been made.

## Experimental method

Surface soils were obtained from the following three areas in New South Wales:

(1) *Turramurra*. A virgin bushland soil derived from Hawkesbury sandstone; pH *c.* 5·5.

(2) *Pennant Hills*. Soil from a chicken-run established for 10 years, derived from Wianamatta shale; near-neutral.

(3) *Flood channels of the R. Nepean, near Windsor*. A coarse gravel very low in organic matter and subject to extremely high insolation and to river-washing in times of high flood; near-neutral.

Human hair, taken from a long uniform hank, was lightly rinsed in water, sterilized with propylene oxide and cut into pieces 2–5 mm. long with sterile scissors. A thin layer was placed on the bottom of a sterile Petri dish which was then half-filled with the moist soil to be tested, the soil and hair being sufficiently though not repeatedly mixed together. A light layer of hair was then dusted on to the soil surface, the Petri dish lid replaced and the whole allowed to rest on the laboratory bench. Four such 'soil-hair plates' of each soil were prepared. Throughout the experiment the soil was kept sufficiently moist by the addition of sterile water to maintain a saturated atmosphere above it. The hair was sampled daily at first and later at longer intervals, six pieces of hair being taken at random from amongst those lying on the surface of each plate on each occasion. The hair pieces were then placed on potato-dextrose agar in a Petri dish and incubated at 25° C., preliminary experiments having shown that this agar was as satisfactory as Sabouraud's glucose agar or Warcup's agar for this purpose. The plates so prepared were examined at frequent intervals and the fungi were either identified from these plates directly or from subcultures from them.

It was hoped that this technique would have the following advantages:

(1) The majority of the hair pieces dusted on to the soil surface lay, in fact, on other pieces of hair or only very lightly on the soil itself. When removed for sampling, each hair would therefore most likely yield fungi which had actively grown onto the hair, the chances of contamination with spores lying *dormant* in the soil having been reduced to a minimum. This was borne out by the sterility of hairs plated-out a few minutes after being dusted onto the soil.

(2) The ease of handling and sampling the hair made it possible to sample large numbers of pieces over a considerable period, thereby showing something of the succession of fungi occurring.

(3) The resistance of the hair for many days to total destruction, although individual pieces were so small (volume $< 0{\cdot}05$ mm.$^3$), was a great advantage in the prolonged investigation of a micro-environment.

(4) Many fungi sporulated on the hair surface, so that direct observations could be used as a check on the results of isolations.

## The fungi isolated from hair

The fungi isolated, together with their frequencies, are listed in Table 1, whilst an arbitrary visual assessment of frequency by observing sporulation in situ on the hair is given in Table 2. Some of the more important features will now be considered.

Table 1. *Fungi isolated from hair, with frequency of isolation*

| | Turra-murra soil | Pennant Hills soil | Nepean soil |
|---|---|---|---|
| *Absidia orchidis* (Vuillemin) Hagem | . | 6 | . |
| *Aleurisma carnis* (Brooks & Hansford) Bisby | . | 1 | . |
| *Alternaria* B34 | . | . | 18 |
| *Alternaria* D43 | . | . | 1 |
| *Alternaria* T91 | . | 1 | . |
| *Arachniotus* sp. | . | * | . |
| *Aspergillus terreus* Thom | 1 | . | 1 |
| *Botryotrichum piluliferum* Sacc. & Marchal | . | 2 | . |
| *Candida* spp. | . | 9 | 1 |
| *Cephalosporium* B89 | . | . | 2 |
| *Cephalosporium* E79 | 1 | . | . |
| *Chaetomium cochlioides* Palliser | 23 | . | 20 |
| *C. funicola* Cooke | 8 | . | * |
| *Chaetomium* G8 | 8 | . | . |
| *Cladosporium herbarum* (Persoon) Link | 1 | . | . |
| *Cladosporium* B1 | . | . | 1 |
| *Entomophthora coronata* (Cost.) Kevorkian | 1 | 1 | . |
| *Cunninghamella elegans* Lendner | . | 1 | 9 |
| *Curvularia lunata* (Wakker) Boedijn | . | . | 1 |
| *Cylindrocladium parvum* Anderson | . | 4 | . |
| *Epicoccum nigrum* Link | . | . | 16 |
| *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen | 42 | 66 | 64 |
| *F. roseum* Link emend. Snyder & Hansen† | 10 | 55 | 17 |
| *Fusidium* K75 | 19 | . | . |
| *Gliocladium roseum* (Link) Thom | . | . | 39 |
| *G. vermoeseni* (Biourge) Thom | . | . | * |
| *Gymnoascus gypseum* Nannizzi‡ | 1 | 3 | 1 |
| *Helminthosporium* A21 | . | . | 1 |
| *Helminthosporium* S16 | . | 1 | . |
| *Humicola grisea* Traaen | 8 | . | 29 |
| *H.* ? *fuscoatra* Traaen | 84 | . | 23 |
| *Keratinomyces ajelloi* Vanbreuseghem | 3 | 3 | 2 |
| *Mortierella humilis* Linnemann | 1 | 41 | |
| *M. mutabilis* Linnemann | 91 | 3 | 55 |
| *Mortierella* sect. Polycephala R117 | . | 2 | . |

Table 1 (*cont.*)

| | Turra-murra soil | Pennant Hills soil | Nepean soil |
|---|---|---|---|
| *Mortierella* sect. Pusilla E80 | 5 | . | . |
| *Mortierella* E50 | 3 | 65 | . |
| *Mortierella* R3 | . | 1 | . |
| *Mortierella* R6 | . | 1 | . |
| *Mortierella* S119 | . | 1 | . |
| *Mucor abundans* Povah | . | 26 | . |
| *M. petrinsularis* Naumov | . | . | 1 |
| *M. spinescens* Lendner | . | . | 8 |
| *Oospora* H97 | . | . | * |
| *Papularia sphaerospora* (Pers.) von Höhhel | 1 | . | . |
| *Penicillium commune* (series) | . | 1 | . |
| *P. frequentans* Westling | . | 1 | . |
| *P. funiculosum* Thom | . | . | 1 |
| *P. janthinellum* Biourge | 3 | 118 | . |
| *P. ? janthinellum* (series) T80 | . | 86 | . |
| *P. ? jenseni* Zaleski | . | 1 | . |
| *P. ? lanosum* Westling | 44 | . | 2 |
| *P. lilacinum* Thom | 5 | . | 1 |
| *P. paxilli* Bainier | . | 1 | . |
| *P. pulvillorum* Turfitt | . | . | 1 |
| *P. purpurogenum* Stoll | 1 | . | . |
| *P. spinulosum* Thom | 1 | . | . |
| *P. thomii* Maire | 9 | . | . |
| *P. thomii* (series) | . | 2 | . |
| *P. vermiculatum* Dangeard | 1 | . | . |
| *P. verruculosum* Peyronel | 2 | . | . |
| *Penicillium* B24 | . | . | 7 |
| *Penicillium* J8 | 5 | . | . |
| *Penicillium* J10 | 1 | . | . |
| *Penicillium* K27 | 1 | . | . |
| *Phialophora* R21 | . | 16 | . |
| *Phoma* A101 | . | . | 19 |
| *Phoma* B41 | . | . | 26 |
| *Phoma* C6 | . | . | 20 |
| *Phoma* C16 | . | . | 20 |
| *Phoma* E38 | 3 | . | . |
| *Phoma* K21 | 3 | . | . |
| *Phoma* R14 | . | 2 | . |
| *Pyrenochaeta* A10 | . | . | 27 |
| *Pyrenochaeta* B3 | . | . | 2 |
| *Pyrenochaeta* C63 | . | . | 1 |
| *Pyrenochaeta* E2 | 5 | . | . |
| *Sphaeronema* sp. | . | . | * |
| *Streptomyces* spp. | * | . | * |
| *Syncephalastrum racemosum* (Cohn) Schroeter | . | . | * |
| *Thielavia sepedonium* Emmons§ | . | 3 | . |
| *Thielavia* S26 | . | 3 | . |
| *Trichocladium opacum* (Corda) Hughes | 1 | . | . |
| *Trichoderma viride* Fr. | 3 | 11 | 1 |
| *Trichophyton terrestre* Durie & Frey | . | 4 | . |
| *Verticillium terrestre* (Link) Lindau | 7 | . | . |
| Mycelium sterilium R5 | . | 130 | . |
| Unidentified (Dematiaceae) S40 | . | 4 | . |
| Mycelia Sterilia | 16 | 10 | 12 |
| Total (species) | 40 | 37 | 40 |
| Total (species, with dark or protected spores) | 12 | 4 | 18 |
| Total (isolates) | 422 | 686 | 450 |
| Total (isolates with dark or protected spores) | 145 | 18 | 237 |

* Isolated, but not in main experiment.

† Of these, twenty-five in Pennant Hills and seventeen in Nepean were very close to *F. equiseti* (Corda) Sacc.

‡ The imperfect state (*Microsporum gypseum* (Bodin) Guiart & Grigoraki) was alone produced by the isolates from Pennant Hills and Nepean soils.

§ Imperfect state only (Emmons, 1932)

### *Species present on hair from all soils*

*Cunninghamella elegans*, *Fusarium oxysporum*, *F. roseum*, *Gymnoascus gypseum* (imperfect stage = *Microsporum gypseum*), *Keratinomyces ajelloi*, *Mortierella mutabilis*, *Penicillium lilacinum*, *Streptomyces* spp. and *Trichoderma viride* were present on hair from all soils and can presumably be considered as typical constituents of the microflora, although of varying importance. *P. lilacinum* and, to a less degree, *C. elegans*, are certainly underrepresented in the isolation results, as is shown by the results of visual observation (Table 2). *T. viride*, although isolated from hair from all three soils, is comparatively rare and does not appear to be of importance, in marked contrast with its

reported abundance on other substrates. The frequency of *G. gypseum* and *K. ajelloi* is to be expected, considering the substrate used, and is in accord with the work of others.

*Species present on hair from only two soils*

*Aspergillus terreus*, *Chaetomium cochlioides*, *C. funicola*, *Entomophthora coronata*, *Curvularia lunata*, *Humicola ? fuscoatra*, *Humicola grisea*, *Mortierella humilis*, *M.* E50, *Penicillium janthinellum* and *P. ? lanosum* were isolated from two soils and can therefore presumably be considered as of widespread occurrence, even if in small numbers in certain cases, in the soil–hair environment. Of these, *C. cochlioides* would appear to be the most important,

Table 2. *Fungi seen sporulating on hair**

| | Turramurra soil | Pennant Hills soil | Nepean soil |
|---|---|---|---|
| *Alternaria* B34 | . | . | +++ |
| *Arachniotus* sp. | . | + | . |
| *Aspergillus terreus* | . | . | + |
| *Chaetomium cochlioides* | ++ | . | ++ |
| *C. funicola* | + | . | + |
| *Cunninghamella elegans* | + | + | ++ |
| *Curvularia lunata* | + | . | ++ |
| *Epicoccum nigrum* | . | . | ++ |
| *Fusarium* spp. | ++++ | ++++ | ++++ |
| *Gliocladium roseum* | . | . | ++ |
| *Gymnoascus gypseum* | ++++ | +++ | ++++† |
| *Helminthosporium* A21 | . | . | + |
| *Humicola* spp. | ++ | . | ++ |
| *Keratinomyces ajelloi* | ++ | ++‡ | ++ |
| *Mortierella* spp. | ++++ | ++ | +++ |
| *Mucor* sp. | . | . | + |
| *Paecilomyces* sp. | . | § | . |
| *Penicillium lilacinum* | ++ | + | ++ |
| *Penicillium* spp. | +++ | +++ | +++ |
| *Phoma* spp. | + | . | ++ |
| *Pyrenochaeta* spp. | + | . | ++ |
| *Streptomyces* spp. | ++ | +++ | ++ |
| *Thielavia sepedonium* | . | ++‖ | . |
| *Thielavia* S26 | . | + | . |
| *Trichoderma viride* | + | + | . |
| *Trichophyton terrestre* | . | +++ | . |

* Frequency, at maximum colonization irrespective of time, is indicated thus: +, uncommon; ++, fairly common, found on soil-hair plate without difficulty; +++, common; ++++, abundant, on great majority of hairs.

† Only the imperfect state, *Microsporum gypseum*, was seen on Nepean plates.

‡ The unnamed perfect state (Dawson & Gentles, 1959) was seen on Pennant Hills plates.

§ Seen, but not in main experiment.

‖ Only the asexual state was seen.

although *H. ? fuscoatra*, *H. grisea* and the two *Mortierella* spp. were also very common in one of the two soils involved. *C. lunata* is almost certainly underrepresented in Table 1 (see Table 2). The isolation of *E. coronata* is of interest, being the second reported isolation from soil (Miller, Giddens & Foster, 1957). The extreme scarcity of *Aspergillus* spp. is noteworthy, especially in view of their reported frequency in the general soil flora of warm climates (but see Table 3).

*Species frequently isolated, but from one soil only*

*Alternaria* B34, *Epicoccum nigrum*, *Fusidium* K75, *Gliocladium roseum*, *Mucor abundans*, *Penicillium* T80, *Phialophora* R21, *Phoma* spp., *Pyrenochaeta* spp., *Thielavia sepedonium*, *Trichophyton terrestre* and the unknown R5 were all common on hair, but from one soil only. Of these, *T. terrestre* and *T. sepedonium* are, by the evidence of their sporulation, much underestimated in Table 1. It is to be presumed that all these fungi are either not present in the other two soils, or being present, are unable to colonize the hair in the environment provided by those soils. An example of this latter possibility is provided by *Humicola grisea*, recovered from the Pennant Hills soil (Table 3) but not isolated from hair from that soil.

Table 3. *Genera represented in isolates from soil, using the soil-plate technique, with frequency of isolation*

| | Turra-murra soil | Pen-nant Hills soil | Ne-pean soil | | Turra-murra soil | Pen-nant Hills soil | Ne-pean soil |
|---|---|---|---|---|---|---|---|
| *Alternaria* Wallr. | 1 | 3 | 1 | *Phialophora* Medlar | . | 1 | . |
| *Aspergillus* Fr. | 2 | 3 | 2 | *Phoma* Sacc. | 9 | 2 | 96 |
| *Botryotrichum* Sacc. & Marchal | . | 9 | . | *Podosporium* Schw. | . | . | 1 |
| *Candida* Berkh. | . | 12 | . | *Pullularia* Berkh. | . | 2 | 13 |
| *Cephalosporium* Corda | . | . | 2 | *Pyrenochaeta* de Not. | . | . | 3 |
| *Chaetomium* Fr. | 22 | 1 | . | *Rhizopus* Corda | 22 | . | 1 |
| *Cladosporium* Fr. | . | 6 | . | *Scopulariopsis* Bain. | . | 1 | . |
| *Cunninghamella* Matr. | 142 | 1 | 2 | *Stemphylium* Wallr. | . | 2 | . |
| *Epicoccum* Wallr. | . | 2 | . | *Syncephalastrum* Schroet. | . | . | 28 |
| *Fusarium* Fr. | 19 | 120 | 23 | *Thielavia* Zopf | . | 2 | . |
| *Fusidium* Fr. | 6 | . | . | *Trichoderma* Fr. | 14 | 37 | 1 |
| *Helminthosporium* Fr. | . | 8 | 10 | *Verticillium* Wallr. | 86 | 2 | 3 |
| *Humicola* Traaen | 5 | 10 | 27 | Mycelium sterilium R5 | . | 9 | . |
| *Mortierella* Coemans | 5 | 20 | . | Unidentified and Mycelia Sterilia | 3 | 8 | 1 |
| *Mucor* Fr. | . | 74 | . | | | | |
| *Nigrospora* Zimm. | . | 1 | . | | | | |
| *Penicillium* Fr. | 160 | 79 | 37 | Total (isolates) | 496 | 416 | 251 |
| *Pestalotia* de Not. | . | 1 | . | No. plates prepared | 12 | 10 | 12 |

*Other species of interest*

Other species, although rarely recorded from the hair, are of interest for other reasons. Thus *Aleurisma carnis*, *Cylindrocladium parvum* and *Trichocladium opacum* would appear not to have been previously recorded from soil. The perfect state (*Gymnoascus gypseum*) of *Microsporum gypseum* was rediscovered during the experiments (Griffin, 1960*a*, *b*). In subsidiary experiments with other soils from N.S.W., the following fungi were obtained from hair: *Basidiobolus ranarum*, *Cylindrocarpon radicicola*, *Cylindrocladium scoparium*, *Shanorella spirotricha* (from soil from burrow of Little Penguin, Five Islands, near Port Kembla—the first record outside the U.S.A. and the first from soil (Benjamin, 1956)) and *Peritrichospora lacera* (from intertidal sand, Manly).

*Differences between populations from the three soils*

Certain differences between the populations from the Nepean and the other two soils may be attributed to the exposure of the former to extremely high temperatures and insolation, the 'soil' being in fact a light-coloured river-washed gravel in an area frequently experiencing shade temperatures in excess of 90° F. It is therefore not unexpected that genera with dark spores, e.g. *Alternaria*, *Epicoccum*, *Humicola*, or with spores enclosed in a perithecium (*Chaetomium*) or pycnidium (*Phoma*, *Pyrenochaeta*) should be numerous (eighteen out of forty species, excluding Mycelia Sterilia) and that over half the colonies isolated should fall into these categories. From the Pennant Hills soil, such species were very rare, whereas the Turramurra soil was intermediary. The Nepean soil is also noteworthy for the remarkably few *Penicillium* colonies derived from it, again in marked contrast to the other soils. The Pennant Hills soil, with its high intake of keratin from chicken feathers, yielded five keratinolytic species in contrast to the two species of the other soils.

## The succession during colonization

The experiments were sampled frequently until approximate stability had been reached and at least half of the hairs were colonized by, or were in the process of colonization by, keratinolytic fungi. The discrepancy in times for this state to be produced can be largely attributed to the different laboratory temperatures at different times of year (Turramurra—26 days, Feb.–Mar., summer; Pennant Hills—43 days, Oct.–Nov., spring; Nepean—57 days, Aug.–Oct., winter). The more important data concerning the succession of fungi, as derived from the frequency of isolation from hair, are presented in Figs. 1–3, where 'Day 1' represents the sampling immediately after the initiation of the experiment, and so on. In certain cases, the various species within a genus behaved in the same way and the histogram is given for the genus as a whole.

In the interpretation of the data two problems arise. The first, as mentioned above, is due to the under-representation of certain fungi in the isolation experiments but it is possible to correct for this error to a considerable degree by means of observations on the frequency of occurrence of fructifications on the hair. This problem is known to apply particularly to the keratinolytic fungi and to *Penicillium lilacinum*. The second problem derives from the difficulty of differentiating, amongst the species isolated at any given time, between those active on the hair and those once active but now largely dormant in the form of spores or 'resting-mycelium'. Once again, evidence of intense sporulation is of value, for this probably signifies that the peak of active mycelial extension in that area is past (Hawker, 1957).

In general, there seems little doubt that a steady increase in frequency of isolation reflects a true increase in colonization and that the majority of the isolates obtained during this period would have been derived from active mycelium. A steady frequency of isolation reflects that maximum colonization has occurred and is frequently accompanied by intense and

widespread sporulation; genera producing simple fructifications, e.g. *Penicillium*, *Fusarium*, sporulated earlier in the period than those producing complex ones, e.g. *Chaetomium*, *Phoma*. At the beginning of this period of

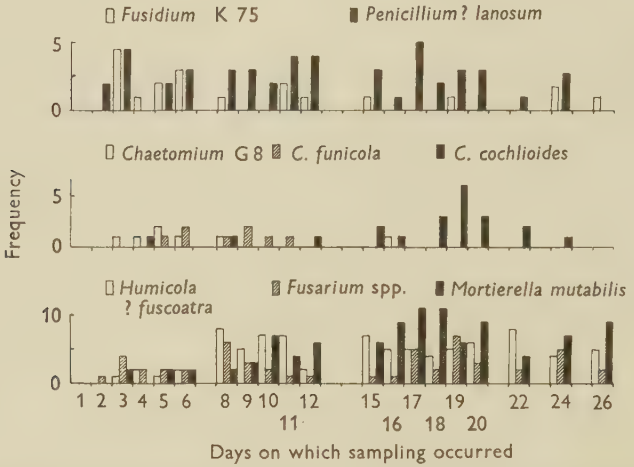
Fusidium K 75
Penicillium? lanosum
Chaetomium G 8
C. funicola
C. cochlioides
Frequency
Humicola ? fuscoatra
Fusarium spp.
Mortierella mutabilis
1 2 3 4 5 6 8 9 10 11 12 15 16 17 18 19 20 22 24 26
Days on which sampling occurred

Fig. 1. The frequency of isolation of certain fungi from hair in contact with Turramurra soil.

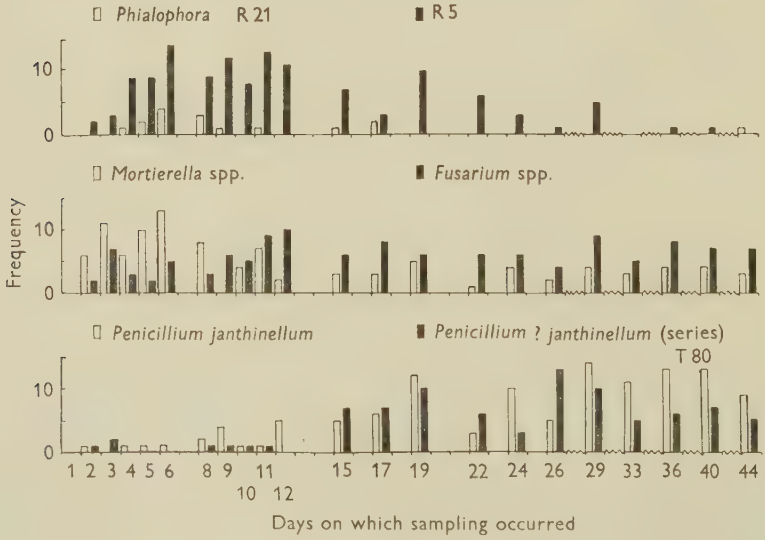
Phialophora R 21
R 5
Mortierella spp.
Fusarium spp.
Frequency
Penicillium janthinellum
Penicillium ? janthinellum (series) T 80
1 2 3 4 5 6 8 9 10 11 12 15 17 19 22 24 26 29 33 36 40 44
Days on which sampling occurred

Fig. 2. The frequency of isolation of certain fungi from hair in contact with Pennant Hills soil.

steady frequency, it seems probable that many of the isolates will have been derived from the active mycelium but that with an increase in time, many will be from spores or other dormant structures. The latter supposition will be particularly likely in the case of fungi which sporulate profusely, and of which the spores germinate easily and the hyphae then grow rapidly.

Some, but not all, species show an eventual decline in frequency of isolation and this is probably due in many cases to the fact that the colonies are now dormant, and on the agar plate fail to grow out before they are swamped by other still active species, or by those with speedier germination and growth. In certain cases, however, there seems no doubt that the species has been actually killed by later colonizers. This is indicated by the dramatic decline of the unidentified R 5, which was the fastest growing of any isolate obtained in the whole series of experiments. Had it survived, it would certainly have outgrown any other competing colony. This case is considered further below. Whatever the reason, a period of declining frequency of isolation probably reflects a similar decline in mycelial activity on the hair.

The main features associated with the succession on the three soils will now be considered.

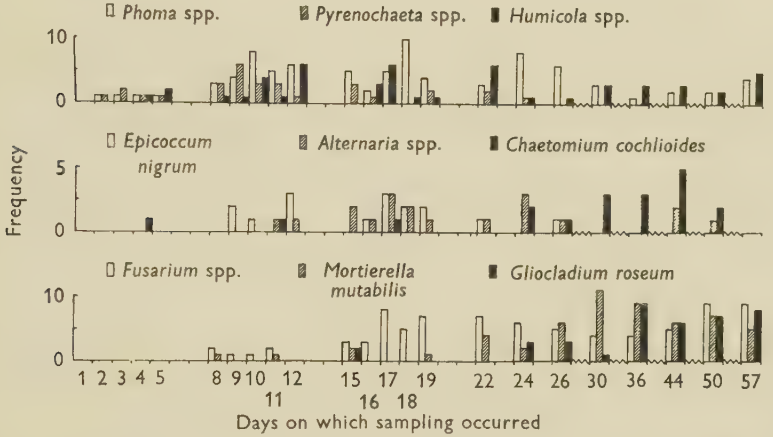


Fig. 3. The frequency of isolation of certain fungi from hair in contact with Nepean soil.

*Turramurra soil.* The total process of colonization was quicker than in the other two soils, no doubt due to the higher temperatures prevailing, and all the hair was probably colonized by some fungus soon after the eighth day. Many fungi took part in the initial colonization but subsequent behaviour differed. Thus the frequency of isolation of *Fusidium* K 75, *Fusarium* spp. and *Penicillium ? lanosum* remained relatively constant (probably due to the speedy germination of spores in the later stages) whilst that of *Humicola ? fuscoatra* and *Mortierella mutabilis* increased, the former until the eighth day, the latter till the eighteenth day, to a steady maximum. The pattern of recovery of the three *Chaetomium* spp. is of interest in that they show complementary patterns, *C.* G 8 being largely replaced by *C. funicola* which, in its turn, was replaced by *C. cochlioides*. *Penicillium lilacinum* was first isolated on the ninth day and was often seen sporulating thereafter, especially in the later phases, as were *Streptomyces* spp. The final keratinolytic colonizers were first noted on the seventeenth day, when sporulation of *Keratinomyces ajelloi* commenced. *Microsporum gypseum*

was later in appearance but ultimately colonized a far greater percentage of hairs than *K. ajelloi*.

*Pennant Hills soil*. The speed of early colonization was far faster than in either of the other soils, virtually all the hairs being colonized by the third day. All major non-keratinolytic colonists were present within the first few days. The frequency of isolation then remained uniform throughout the experiment, except for *Mortierella* spp. and *Phialophora* R21 which decreased after the eighth day, and R5 which gradually decreased after the thirteenth day, coinciding with the increase of *Penicillium* T80 and *P. janthinellum*. Sporulation of the first keratinolytic fungus, *Thielavia sepedonium* (Emmons, 1932) occurred on the seventeenth day, soon to be followed by *Keratinomyces ajelloi*, *Microsporum gypseum* and *Trichophyton terrestre*, almost simultaneously, and finally by an undescribed *Arachniotus* sp. *M. gypseum* and *T. terrestre* finally occupied the greatest areas followed, in order, by *K. ajelloi*, *T. sepedonium* and *Arachniotus* sp. Fructifications of *Penicillium lilacinum* were very occasionally seen during the latter half of the experiments and *Streptomyces* spp. were common at the same time.

The spectacular decrease of R5, *Mortierella* E50 and *Phialophora* R21 at the same times as the increase of *Penicillium janthinellum* and *Penicillium* T80 was further investigated. It was found that the growth of the three first-named fungi on an agar plate was severely restricted by the presence of a colony of *Penicillium* T80 (probably a member of the *janthinellum* series) but was unaffected by *P. janthinellum*. That the antibiotic produced was similar to griseofulvin was indicated by the morphological response of the hyphae and by the fact that *Pythium ultimum* was unaffected (Brian, 1949). The *Penicillium* colony eventually partially overgrew those of the other three species, and enveloped and penetrated their hyphae with its own. There would therefore seem to be strong evidence that these fungi, at least, were inhibited and probably killed by a later colonizer, *Penicillium* T80. This situation is being further investigated.

*Nepean soil*. The first major colonizers were most unexpectedly species of *Phoma* and *Pyrenochaeta*, quickly followed by *Humicola* spp. and then by *Epicoccum nigrum* and *Fusarium* spp. Of these, only *Fusarium* spp. maintained a high frequency of isolation after 27 days, no doubt reflecting their heavy sporulation, and speedy germination and growth from spores, rather than continued activity. These fungi were followed, in order, by *Alternaria* B34, *Mortierella mutabilis* and *Gliocladium roseum*, whilst *Chaetomium cochlioides* became more prominent in the middle and later stages. The first keratinolytic fungus, *Keratinomyces ajelloi*, was seen sporulating after 38 days and it gradually increased, until after 70 days it had colonized about one-quarter of the hairs. *Microsporum gypseum* was first isolated after 38 days but was slower to sporulate. However, by 70 days, it was far commoner on the hair than was *K. ajelloi*. *Penicillium lilacinum* was frequently seen sporulating on the hair after 36 days and colonies of *Streptomyces* spp. were often seen in these later stages. All the hair was colonized by some fungus by the seventeenth day.

The succession therefore reflects, as does the flora revealed as a whole, the harsh environment of the Nepean sand, the first colonizers all being dark-spored, or with spores produced within protective structures. Such

success in early colonization presumably reflects the relative abundance of spores in the soil and their resistance to destruction by heat and light, as well as the speed of hyphal growth. The light-spored species, *Fusarium* spp., *Mortierella mutabilis* and *Gliocladium roseum*, largely comprised a later group, having possibly been restricted at first by poverty of inoculum in the soil. It is, however, of interest to note that these latter results have a certain similarity to those obtained by Stenton (1958) in his study of the colonization of pea roots. There he found that *Fusarium* spp. were present at a comparatively steady level throughout the experiment, that *Mortierella* sp. was most frequent in the middle stages and that *Gliocladium roseum* had a marked ability for late colonization, competing with previously established species.

### Fungi isolated using soil-plates

The experiments described above provide an assessment of the number and variety of colonies involved in the colonization of hair. It therefore seemed of interest to investigate the same soils using one of the often-used methods for the study of the general soil fungal flora, so that a comparison between the results of the two techniques could be made. For this purpose, Warcup's (1950) soil-plate method was employed, and the results are presented in Table 3. The survey using this technique was not exhaustive and the isolates have only been identified to generic level. The accuracy of any comparison is also limited by the different times of the year at which the soils were collected.

Comparing Tables 1 and 3, it becomes apparent that the majority of the important early colonizers of the hair surface are represented on the soil-plates, whereas the more specialized keratinophilic fungi are not. Such a difference is to be expected in view of the poor competitive saprophytic ability of the latter on the normal agar media and it can be regarded as a particular example of a rule that generalized methods for sampling the flora will not reveal, at least in their true importance, those fungi which are ecologically restricted to unusual substrates in the soil, in this case keratin. The soil basidiomycetes have long been recognized as a similar group.

A second point of difference can be attributed to the difference in origin of the isolates in the two series, i.e. each isolate derived from a discrete colony on hair in the first and from an assortment of spores and hyphae, dormant or active in soil, in the second. As has often been pointed out, the soil-plate method will thus favour the isolation of fungi which sporulate profusely (although even then not as much as will the dilution-plate method) as against those with poor or no sporulation. This is certainly borne out by the predominance of such genera as *Cunninghamella*, *Mucor*, *Penicillium*, *Trichoderma* and *Verticillium* and the comparative dearth of *Mortierella* (poorly sporulating) and R 5 (sterile) on the soil-plates, despite the fast growth of the latter, and this is not paralleled in the isolations from hair.

## Discussion

It cannot be claimed that these experiments lead to any new concepts in the field of soil ecology but they do provide further evidence in support of various existing ideas and principles. Thus the experiments have added confirmation to the view that a given micro-environment, in this case 'hair-in-contact-with-soil', does have a characteristic fungal flora and succession. It can be expected that this environment, like others, will first be occupied by fungi of high competitive saprophytic ability able rapidly to utilize the less complex nutrients of the substrate. *Fusarium* spp., certain *Penicillium* spp. and various members of the Mucorales are characteristic of this stage. These overlap with or are followed by the second group, of which *Chaetomium cochlioides*, *Gliocladium roseum*, *Humicola* spp. and other *Penicillia* are characteristic. The basis of this stage of the succession is almost certainly complex, but is probably in part linked with an ability to utilize more resistant parts of the substrate, the genera *Chaetomium* and *Humicola* being well-known for their high cellulolytic activity, for instance. The final fungi to appear as active members in the environment are those fungi of low general competitive saprophytic ability but which are able to utilize the final and most resistant part of the substrate. The keratinolytic fungi *Keratinomyces ajelloi* and *Microsporum gypseum* are particularly characteristic.

The broad outlines of the general succession are therefore clear, but the fate of individual colonies which are being succeeded is uncertain. Many certainly sporulate profusely as their peak of activity passes and then survive as dormant spores, the hyphae of the colony being presented as empty tubes to the succeeding fungi. In others, however, much of the protoplasm of the colony is probably destroyed by direct parasitism by a succeeding fungus, as in the case of R 5 and *Penicillium* T 80. It is obvious that there is scope for much future investigation concerning these replacement phenomena, and hair has many advantages for use as the substrate.

It must, however, be obvious when the varying soils and temperatures are considered that there is no one 'soil-hair' micro-environment and that the general similarities of the microflora must be due more to the identity of the substrate and the uniform (high) humidity throughout the experiments than to similarities in the soil moieties of the over-all 'soil-hair' environment. Apart from such factors as pH which might influence the rate of growth of a colonizing fungus, the soil will affect the succession, and thus the hair microflora, in its capacity as provider of the fungal inoculum, and these differences in inoculum will reflect the history of the soil. In the Nepean soil used in these experiments, the past history of high insolation and of river-washing have had a marked effect on the flora of the soil-hair micro-environment, favouring, especially in the early stages of colonization, fungi with dark, or otherwise protected, and long-lived propagules. Similarly, the history of high input of keratinous and nitrogenous waste products from fowls into the Pennant Hills soil has affected the availability of fungi in that soil to provide an enhanced inoculum in the specific micro-environment under consideration. Such

differences would appear, however, only to modify the general substrate pattern of fungal activity, adding or subtracting a few species at various points in a succession which remains fairly predictable.

The very existence of keratinolytic fungi in the great variety of soils which have now been examined by various workers raises a problem of great interest, for these fungi seem to be ecologically restricted to keratin as a substrate in soil and yet it is scarcely believable that the accession of keratin in normal soils should be sufficient to support an almost ubiquitous keratinolytic flora, even allowing for very prolonged periods of dormancy. In soils such as that from Pennant Hills many keratinolytic fungi would be expected but the position with the soil from Turramurra is far different, for there all the keratin must be derived from the wild, and restricted, fauna of birds, marsupials and lizards. An investigation of this aspect of the ecology of keratinolytic fungi would be most interesting.

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## Note added in proof

After examination of an authentic culture of *Thielavia sepedonium* it would appear that the isolates referred to above as *T. sepedonium*, and only found in these experiments in the imperfect state, do not in fact belong to that species. Although the asexual stages of the two fungi are practically identical, the colony appearance is very different and the culture of *T. sepedonium* has proved to be non-keratinolytic. The identity of the strongly keratinolytic fungus is being studied further.

*Trans. Brit. mycol. Soc.* **43** (4), 597–602 (1960).

# *SAPROLEGNIA ASTEROPHORA* DE BARY (1860)*

By M. W. DICK

*Queen Mary College, University of London*

(With 3 Text-figures)

*Saprolegnia asterophora*, isolated from soil, is described and illustrated; attention is drawn especially to the oospore structure.

## *Saprolegnia asterophora* de Bary (1860)

*Mycelium* diffuse, reaching about 3 cm. diam. on hemp seed in a Petri dish half filled with sterile, glass-distilled water at 18–20° C.; principal hyphae thin flaccid, often undulating, 20–30 (40) $\mu$ diam. at the base, but elsewhere mostly approx. 12 $\mu$ diam. *Zoosporangia* abundant, clavate, dimensions (100 measured) in the range (170) 333–503 (796) $\mu$ by (13) 24 (37) $\mu$, the wall often thickened distally with a thick funnel-shaped exit papilla; secondary renewal either by internal proliferation (up to 7 times), basipetal succession (up to 5) or sometimes lateral; complex sporangial systems exhibiting all types of renewal may occur, especially in rough culture. *Gemmae* absent. *Oogonia* moderately abundant, single on long lateral branches or sometimes (up to 15%) inside the sporangia; rarely sporangia may be formed below the oogonia (Fig. 2G); oogonia spherical, diam. (based on measurements of 300 oogonia) in the range (31) 45–52 (76) $\mu$ including ornamentations, less frequently longer than broad, when they may possess an apiculus; with or without a neck, basal septum with or without an internal projection; wall densely ornamented with short, blunt or rounded papillae; pits absent. *Antheridia* very rare in this isolate, when present androgynous (monoclinous) or semi-hypogynous, the antheridial cell short-clavate, attached by its apex to the oogonium. Fertilization tubes not observed. *Oospheres* usually one (3% with more than one oosphere), maturing, filling the oogonium (apart from the papillae). *Oospores* subeccentric; diam. in the range (19) 30–35 (49) $\mu$. Germination not observed.

Previous reports have come from de Bary (1881, 1888), Trelease (in Humphrey, 1893), Fischer (1892), Istvánffi (1895), Petersen (1910), Minden (1912), Coker, (1923), Häryén (1928), Apinis (1930), Lund, (1934), Ivimey Cook & Morgan (1934), Höhnk (1935), Forbes (1935), van Beverwijk (1948) and Beneke (1948). All have regarded this species as rare, though it has been found in America and various countries of Europe; I have found it not uncommonly in damp, strongly acid soils. All authors suggest that the sporangia are scarce, but this is not true for my isolate, which appears extreme in two other features, though in dimensions it agrees well with all other descriptions. Most authors suggest that the frequency of the antheridia is variable, but only Coker has

* Part of a thesis approved for the degree of Ph.D. in the University of London.

Fig. 1. *S. asterophora*. A–D, types of sporangial renewal: A, C, internal proliferation; B, lateral renewal; D, basipetal succession. E–I, details of sporangia. E–H, thickened funnel-shaped exit papillae; E–G, include encysted zoospores; G, I, sporangial bases. B, I, note the wide angle of lateral renewal; G, a complex arrangement of sporangia and an oogonium. A–D, scale '*a*'; E–I, scale '*b*'.)

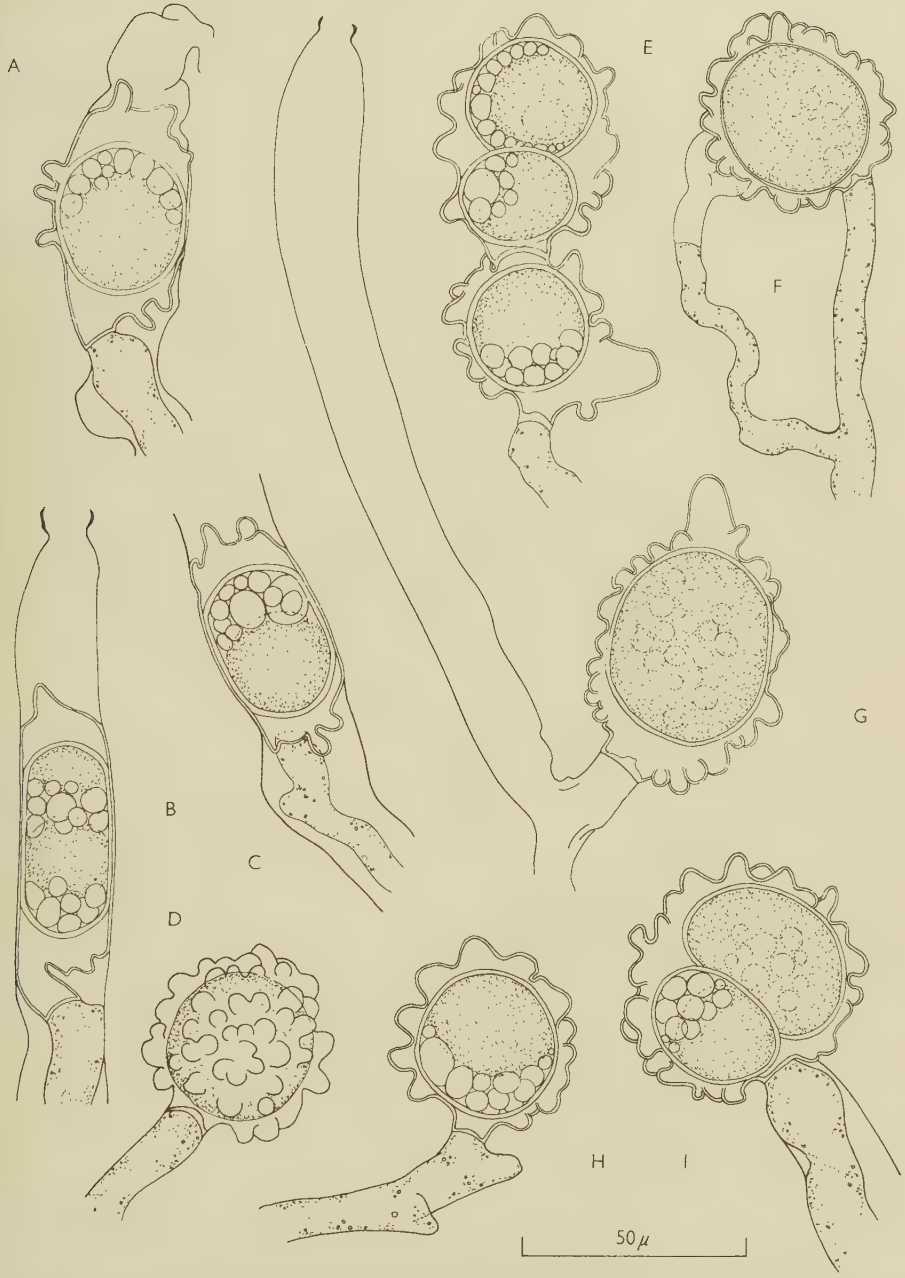


Fig. 2. *S. asterophora.* A–C, I, oogonia within or proliferating through a sporangium; G, sporangium beneath an oogonium, the oogonium somewhat elongate, with an apiculus; D, surface view of an oogonium showing ornamentations; E, I, oogonia containing two oospores; E, two oogonia in series; F, oogonium with an antheridium applied by its apex; A–C, E, H, I, mature subeccentric oospores; B, compound oospore.

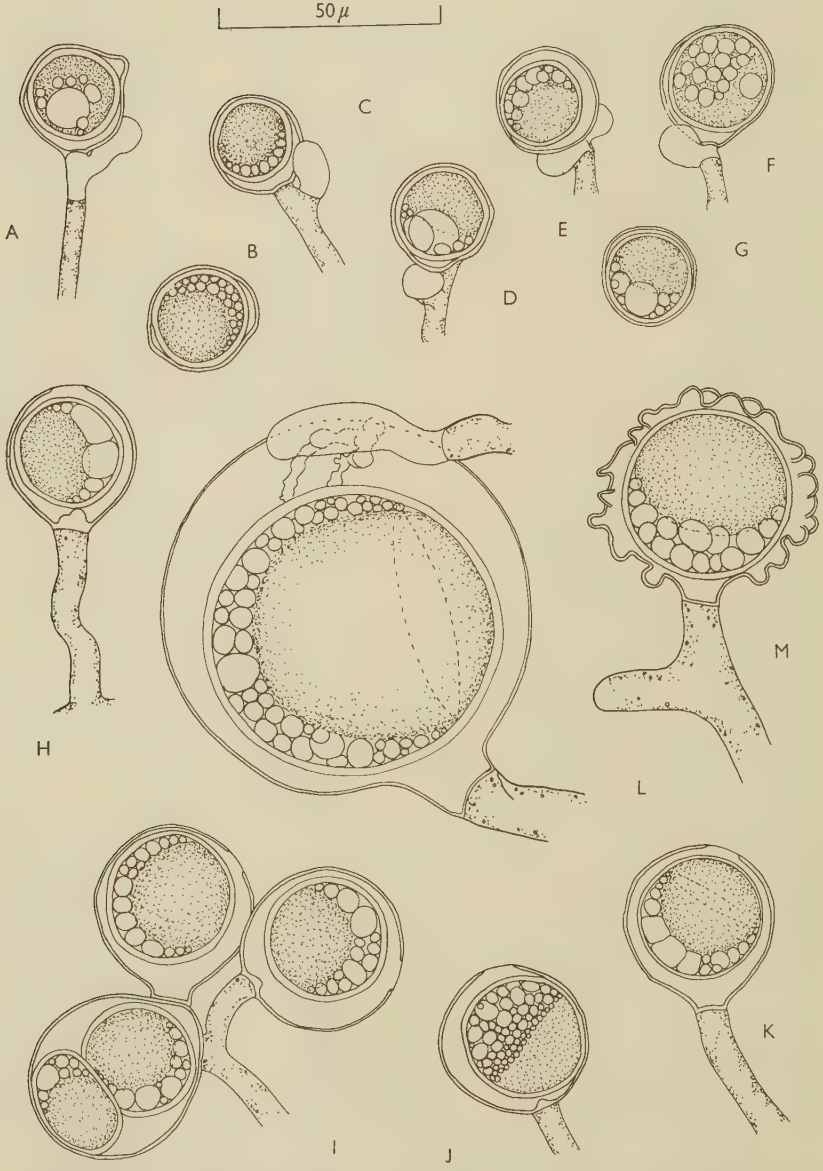


Fig. 3. A–G, mature oospores of *Pythiopsis cymosa*; H–K, mature oospores of *Isoachlya eccentrica*; L, mature oospore of *Calyptralegnia achlyoides*; M, mature oospore of *Saprolegnia asterophora*. (All show a subeccentric structure.)

recorded the fact that the antheridia may be almost entirely absent at 'low temperatures', but in my isolate antheridia were scarce at room temperature as well as at 3–8° and 10–12° C. However, when present they resemble the illustrations of de Bary (1860), Istvanffi (1895) and Coker (1923).

The most interesting feature of this isolate is the oospore structure, oil droplets of various diameters forming a 'cap' over the cytoplasm; this 'cap' may be extensive enough to invest the cytoplasm almost completely. It is proposed that a new term 'subeccentric' be used in conjunction with the existing terms of description for oospore structure, namely centric, subcentric and eccentric, to describe this type of oospore structure which is not unique to this isolate, being found in *Pythiopsis cymosa* (Fig. 3A–G), similarly figured in de Bary (1888) and Coker (1923); *Calyptralegnia achlyoides* (Fig. 3L) but described as centric (Coker, 1923) and subcentric (Forbes, 1935); *C. ripariensis* (Höhnk, 1953) and *Isoachlya eccentrica* (Fig. 3H–K) which had previously been described and illustrated (Coker, 1923) as truly eccentric. In all the isolates of these species I have examined, this structure does not change to an eccentric type with age.

There is no previous report of such an oospore structure in *S. asterophora*. De Bary described it as centric, and Coker and Beneke as subcentric. Coker gives an illustration of a normal subcentric oospore.

It should be stated that Johannes (1953) has transferred *S. asterophora* to his new genus *Cladolegnia*, which he regards as intermediate between *Saprolegnia* and *Leptolegnia*. The genus is characterized by oogonia with 1–3 oospores.

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# *DACTYLELLA ARNAUDII* SP.NOV.

By A. S. YADAV

*Imperial College, London*

(With Plate 22 and 1 Text-figure)

*Dactylella arnaudii* sp.nov. found on dead stems of *Heracleum sphondylium* L. is described and compared with closely related species. *Monacrosporium* Oudemans and *Dactylina* Arnaud are considered to be synonyms of *Dactylella* Grove.

**Dactylella arnaudii** sp.nov. (Text-fig. 1, Pl. 22)

Coloniae irregulares, effusae, tenues, albae. Mycelium superficiale, ex hyphis ramosis, septatis, hyalinis, levibus, 1–1·5 $\mu$ crassis compositum. Conidiophora singula vel fasciculata ex apice lateribusque hyphorum oriunda, erecta vel ascendentia, primo simplicia, dein apicem versus parce ramosa, hyalina, septata, 23·5–109 $\mu$ longa, 3·3–4·4 $\mu$ crassa. Conidia solitaria in apicibus stipitis ramulorumque oriunda, fere fusiformia, interdum rostrata, hyalina, levia, tenuiter tunicata, 3–12 septata, 29·5–92 $\mu$ longa, 3·3–9·9 $\mu$ crassa.

In caulibus emortuis Heraclei sphondylii L., St Albans, 25 VIII 1958, A. S. Yadav, Herb. I.M.I. No. 77850, typus.

The fungus was found in September 1958, whilst the species associated with the decay of stems of *Heracleum sphondylium*, collected from a garden in St Albans, were being studied.

*Colonies*, white, loose and thin, growth over the stem spreading irregularly; *mycelium*, hyaline, thin-walled, branched, 1–1·5 $\mu$ diam.; *conidiophores* hyaline, thin-walled, solitary or in groups, septate, 23·5–109 × 3·3–4·4 $\mu$, terminating in a conidium, then forming a number of small branches (usually 2–4) below; *conidia*, hyaline, thin-walled, 3–12-septate, fusiform, sometimes rostrate, borne singly at the apex of the conidiophore and on the short branches, 29·5–92 × 3·3–9·9 $\mu$.

*Details of 12-day-old colonies grown at 22–23° C.*

On Czapek agar (made with 20 g. agar) 6 mm. diam., off-white, thin; reverse, whitish; sporulation very poor.

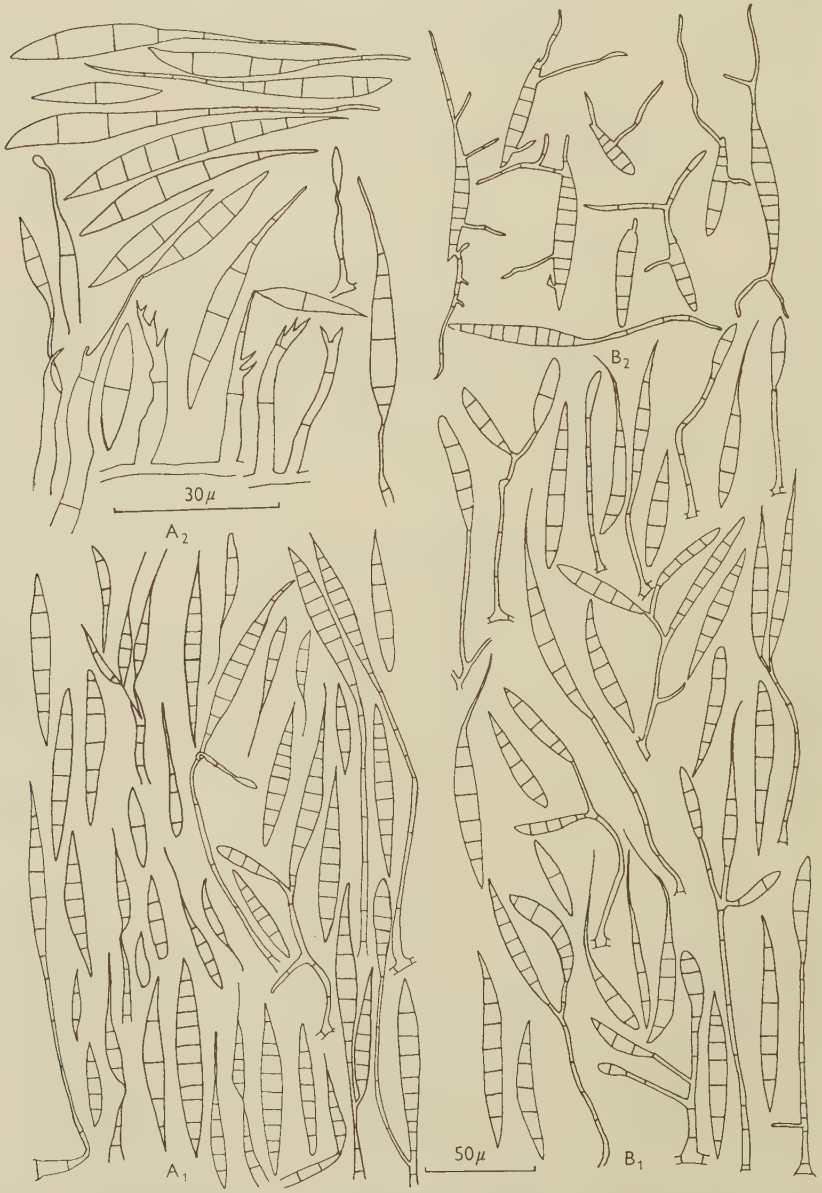
On potato dextrose agar (20 g. agar), 7 mm. diam., off-white, forming a raised woolly cushion; reverse dull white; sporulation good.

On malt agar (30 g. malt extract, 20 g. agar), 7 mm. diam., off-white, forming a more or less raised woolly cushion as on potato dextrose agar; reverse dull white; sporulation good.

Morphological details on malt agar are almost the same as on the natural substratum: conidiophores, 30–118 × 2·2–2·3 $\mu$, branched 2 to 30$\mu$ (rarely up to 50 $\mu$) below the apex; conidia, 28–102 × 4–7·7 $\mu$.

*Germination and development of the conidia*

Conidia germinate readily within 24 hr. in distilled or tap water. Each cell is capable of giving rise to a germ tube. Usually two to three germ-tubes arise from each conidium. Each conidium originates as a swollen tip




Text-fig. 1. *Dactylella arnaudii*. $A_1$, conidia and conidiophores (natural substratum); $A_2$, conidia and conidiophores showing fine prolongations; $B_1$, conidia and conidiophores (culture); $B_2$ germinating spores.

which increases in size, then gradually elongates, becoming septate at the same time.

On a range of media with different C sources growth was good on some. The C source at 1 % w.v. was added to a basal medium with the final composition of 0·2 % $NH_4NO_3$, 0·2 % $MgSO_4.7H_2O$, 0·2 % $K_2HPO_4$, 100 $\mu$g./l. thiamine, 5 $\mu$g./l. biotin, 1 ml./l. of trace element solution (prepared according to Lilly & Barnet, 1951), and 2 % agar. Phosphate was autoclaved separately and added aseptically to the rest of the medium giving a final volume of 25 ml. medium per 10 oz. medical flat. Each was centrally inoculated and incubated at 22–23° C. The results are summarized in Table 1. The fungus grew weakly on purified wood cellulose ('Solka-floc' manufactured by Brown Co., Boston, Mass.), but if this was supplemented by 0·1 % glucose it grew well (better than with either of these C sources separately)

Table 1. *Growth of* Dactylella arnaudii *on media with different carbon sources*

(12-day-old colonies)

| Medium | Diam. in mm. | Appearance | Reverse |
|---|---|---|---|
| Basal (No C) | — | No growth | — |
| Glucose | 35 | White, woolly | Whitish |
| Sucrose | 12 | White, woolly | Whitish |
| Starch | 35 | White, woolly | Whitish |
| Pectin | 35 | White, woolly | Whitish |
| Sodium pectate | 16 | White, woolly | Whitish |
| Cellulose | 30 | White, thin | Whitish |
| Cellulose+0·1 % glucose | 34 | White, woolly | Whitish |
| 0·1 % glucose | 30 | White, very thin | Whitish |

There are marked differences between this species and species with fusiform conidia. Its conidia are narrower than those of *D. minuta* Grove var. *fusiformis* Grove, which measure 60–75 × 7–9 $\mu$ and have 6–8 septa (Grove, 1884), also they are borne on branched conidiophores unlike the simple ones of Grove's fungus. None of the conidia of the latter are described as rostrate.

*D. attractoides* Dreschler has much branched conidiophores and broader conidia 26–76 × 7·2–10·2 $\mu$ (Dreschler, 1943). The conidia are also not rostrate.

*D. arnaudii* resembles more closely a figure given by Arnaud (1952) representing a species of *Dactylina.* He gave no Latin description so that we have little idea of the general morphology and measurements of his fungus. Therefore, my fungus is here treated as a new species and named after Prof. G. Arnaud.

Grove (1884) created the genus *Dactylella* with *D. minuta* Grove, a mucidinous fungus, as its type species, showing the following spore characters '...Sporeae elongateae, solitareae, acrogeneae, pluriseptatatae...'. Oudemans (1885) later erected the genus *Monacrosporium*, also with acrogenous pluriseptate conidia, and placed in it two species, *M. elegans* Oud., with broadly fusiform conidia, and *M. subtilis* Oud., usually taken as typifying the genus, with narrow elongated conidia.

Variation in spore shape in *D. arnaudii* and allied species, however, indicates that spore shape alone is not a sufficiently good character on which to maintain a separate genus.

Arnaud (1952) created the genus *Dactylina* for *Dactylella*-like fungi which have rostrate conidia, but, as recorded above in *D. arnaudii*, some conidia are fusiform while others are rostrate, so that here too the shape of the conidia seems to be an inadequate distinction. Therefore, I would propose that the genera *Monacrosporium* Oud. and *Dactylina* Arnaud should both be treated as synonyms of *Dactylella* Grove.

I wish to express my thanks to Dr M. F. Madelin for kind help and encouragement and to Dr M. B. Ellis of C.M.I., Kew, for valuable advice and helpful criticism and also for the Latin diagnosis.

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## EXPLANATION OF PLATE 22

*Dactylella arnaudii* Yadav

Fig. 1. Conidiophore with a very small lateral branch and conidia (natural substratum). × 560.
Fig. 2. Conidiophores bearing rostrate conidia on fine small branches (natural substratum). × 480.
Fig. 3. Conidiophore with a young conidium at its apex (natural substratum). × 480.
Fig. 4. Mycelium and conidiophores bearing conidia at the apex and at the tips of the small branches (culture). × 480.
Fig. 5. Germinating conidia. × 120.
Fig. 6. Conidiophore bearing young conidia on small branches, below its apical conidium (culture). × 560.

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(*Facing p.* 606)

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# PYRENOMYCETES OF SUGAR CANE AND OTHER GRASSES IN JAMAICA

## I. CONIDIA OF *APIOSPORA CAMPTOSPORA* AND *LEPTOSPHAERIA SACCHARI*

By H. J. HUDSON*

*Botany Department, University College of the West Indies, Mona, St Andrew, Jamaica*

(With Plate 23 and 2 Text-figures)

The conidial state of *Apiospora camptospora* Penzig & Sacc. has been shown by cultural studies to be *Papularia vinosa* (Berk. & Curt.) Mason. The *Phyllosticta* state of *Leptosphaeria sacchari* van Breda de Haan is redescribed and figured.

*Apiospora camptospora* Penzig & Saccardo, *Malphigia*, **11**, 398, 1897. (Text-fig. 1)

*Gymnosporium vinosum* Berk. & Curt., *J. Linn. Soc. Bot.* **10**, 355, 1869.
*Coniosporium vinosum* (Berk. & Curt.) Sacc., *Syll. Fung.* **4**, 244, 1886.
*Melanconium saccharinum* Penz. & Sacc., *Malphigia*, **15**, 398, 1901.
*Papularia saccharina* (Penz. & Sacc.) Höhnel, *S.B. Akad. Wiss. Wien*, **128**, 610, 1919.
*Papularia vinosa* (Berk. & Curt.) Mason, *Mycol. Pap. C.M.I.* **3**, 21, 1933.

Ascocarps are very common on the basal stem internodes of *Sorghum halepense*, *Panicum maximum*, *Pennisetum purpureum*, and *Trichachne insularis* in Jamaica; they also occur, but less frequently, on the leaves of sugar cane (*Saccharum officinarum*) where they are usually confined to the region of the midrib. The following description is based on a collection of *Sorghum halepense* (Herb. I.M.I. no. 76764).

*Ascocarps* are formed in the cortex of the lower internodes of dead culms in uniseriate rows of up to ten or occasionally as many as 40. They raise and split the cortex into longitudinal hysterioid cracks usually up to 2 mm. long but occasionally up to 1 cm. They are densely aggregated and easily visible to the naked eye. The ascocarps, connected and covered by a dark pseudostroma, are initially globose and up to 200 $\mu$ diam., but as they mature they become laterally compressed (Text-fig. 1 A). The shortly stalked *asci* are bitunicate, clavate, tapering to a rounded apex, 84–112 × 14–21 $\mu$, 8-spored and surrounded by numerous filiform pseudoparaphyses which are difficult to discern because of the large number of oil guttules which rapidly fill sections or squashes. The biseriate *ascospores* are hyaline or yellowish, curved pyriform, measuring 32–49 × 9–11 $\mu$, and are 2-celled with a septum 6–8 $\mu$ from the base (Text-fig. 1 C and Pl. 23,

* Now at The Botany School, Cambridge.

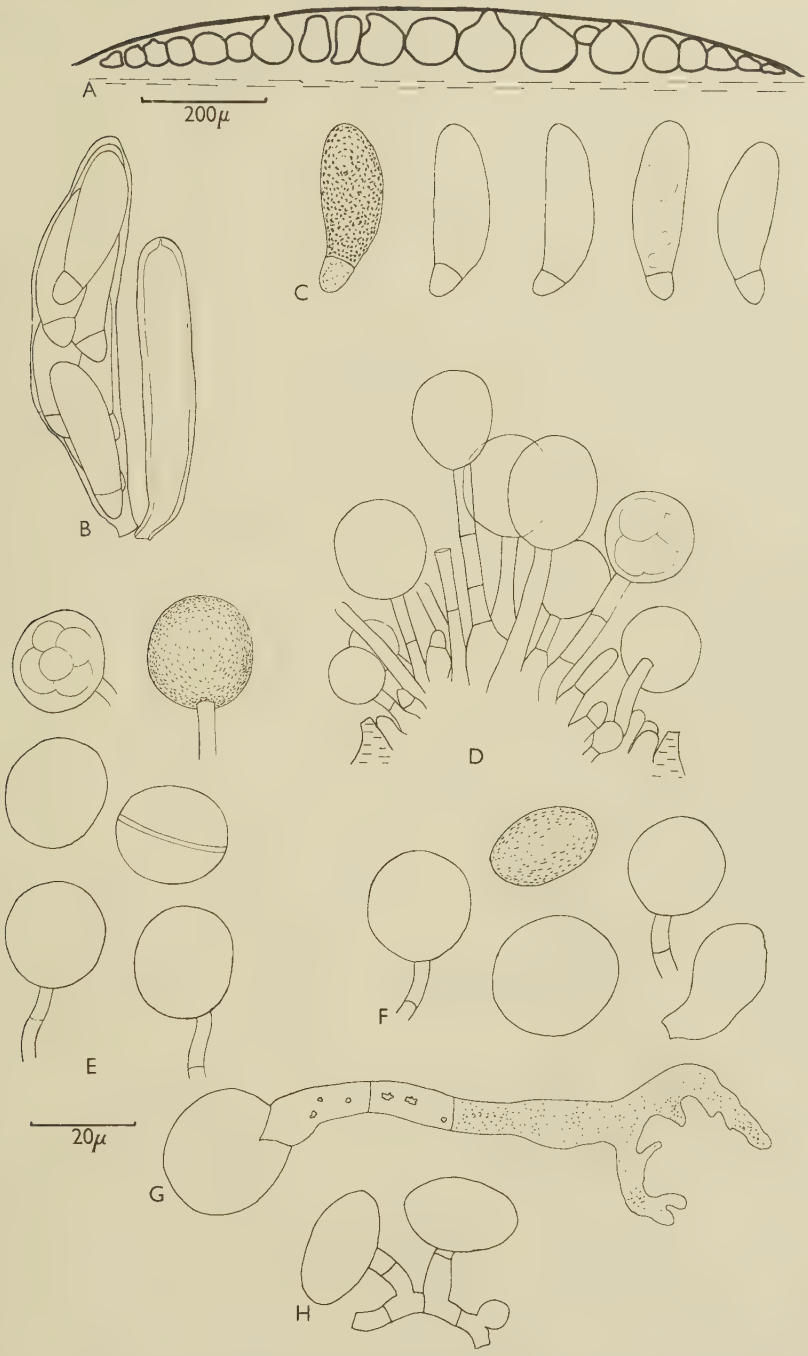
figs. 4, 5). The upper, larger cell has densely granular cytoplasmic contents, often with one or more guttules, whereas the smaller, basal cell usually appears empty.

This specimen has not been matched with authentic material, but it agrees with Penzig & Saccardo's description of the species which is the earliest having an ascospore range comparable to that established on the above hosts (Table 1). Mr E. W. Mason, who kindly examined the Jamaican collections, confirmed that it is an *Apiospora*, whether the type is taken to be *Sphaeria apiospora* Dur. & Mont. (syn. *A. montagnei* Sacc.) or, probably more correctly, *Homostegia striola* Pass. (syn. *A. striola* (Pass.) Sacc.). The fungus was originally described on sugar cane leaves in Java with perithecia in seriate lines or variously congregated, globular, black, scarcely papillate, 180–200 $\mu$ diam., cylindrical asci rounded at the apex, 75–80 × 15–17 $\mu$, and hyaline ascospores curved downwards and with a submedian septum, not constricted, 35–38 × 9 $\mu$. The ascospore measurements given in Table 1 show that the dimensions of the ascospores on sugar cane are somewhat larger than those given for the type collection, and this is true of all the Jamaican collections.

Table 1. *Dimensions* ($\mu$) *of ascospores and conidia of collections of* Apiospora camptospora

| Host | Ascospores on host | Conidia on host | Conidia from cultures of ascospores | Conidia from cultures of conidia |
|---|---|---|---|---|
| *Sorghum halepense* (Herb. I.M.I. no. 76764) | 32–49 × 9–11 | 17–25 × 12–18 | 19–28 × 14–18 | 16–29 × 12–19 |
| *Saccharum officinarum* (Herb. I.M.I. no. 76970) | 39–58 × 10–14 | 21–32 × 19–23 | 20–26 × 15–20 | 19–29 × 15–19 |
| *Panicum maximum* (Herb. I.M.I. no. 76972) | 34–47 × 9–10 | 20·5–28 × 16–19 | 20–26·5 × 14–20 | 21–27 × 14–18 |
| *Arundo donax* | — | 20–28 × 14–19 | — | 20–28 × 17–20 |
| *Pennisetum purpureum* (Herb. I.M.I. no. 76973*a*) | 41–51 × 11–14 | 19–28 × 13–19 | 22–28 × 17–22 | 21–28 × 16–20 |
| *Trichachne insularis* | 38–48·5 × 9–11 | — | 19–28 × 16–19 | — |

Cultures were prepared from single ascospores and asci from all the hosts cited. The initial stages of germination were slow. Each ascospore produced one to six germ-tubes from the larger cell. These germ-tubes were often distorted and developed into appressoria, each in the form of a large much-branched terminal cell full of densely granular cytoplasm. The remaining cells of the germ-tube appeared empty. All isolates on oatmeal agar soon produced a white, cottony mycelium which spread rapidly, turning pinkish orange and later darkening to grey-black when conidia were formed. Conidia arose scattered on the aerial mycelium at first or in irregular clusters. These were soon followed by globose or depressed mycelial cushions, dark in mass but covered with white hyphae and up to 1 cm. diam., which later bore conidia densely aggregated over their entire surface. The conidia (Text-fig. 1E, H) were unicellular, globose or flattened lenticular, smooth, pale brown to purple, 19–28 × 14–19 $\mu$ and were attached to the conidiophore by one point on the periphery. Other spores




Text-fig. 1. *Apiospora camptospora*. A, Ascocarps on *Sorghum halepense* in vertical section; B, asci; C, ascospores; D, section of conidial fructification with developing conidia, mature conidia and conidiophores; E, conidia from an ascospore culture; F, conidia from a conidium culture; G, germinating conidium showing distorted end cell; H, conidia from a culture of a single ascospore from *Saccharum officinarum*.

were irregularly shaped and greatly distorted. Young conidia were hyaline and often guttulate and were produced on undifferentiated aerial mycelium. A single conidium formed terminally at the apex and others were successively produced laterally below this on short, usually unicellular, side branches. Many of the conidia possess a hyaline rim round the periphery. This description agrees with the fungus described by Mason (1933) as *Papularia vinosa* (Berk. & Curt.) Mason.

On the host similar conidia have been found. Conidial fructifications are almost identical with the asco-stromata on the stem. They may appear before the ascocarps or may be scattered amongst them, the latter often arising below the old conidial stromata. They are more clustered towards the nodes and there they are spread laterally rather than elongated as on the internodes. The fructifications start to develop beneath the epidermis which later splits longitudinally to expose the mass of shiny black spores. They appear to the naked eye as black, erumpent, hysterioid masses parallel to the long axis of the culm, as do the ascocarps. The spores appear black in reflected light but yellowish brown in transmitted light when mature. They arise from a superficial network of closely packed hyphae and are produced singly as terminal thallospores at the ends of short, closely packed conidiophores which have thick, brown, transverse septa and often possess a large swollen basal cell (Text-fig. 1 D; Pl. 23, figs. 8, 9). When mature the conidia become detached from the slightly tapering or blunted end of the conidiophore and measure 17–25 × 12–18 $\mu$. The conidia germinate in water or on agar after 24–48 hr. to produce a single rapidly growing germ-tube or an appressorium (Text-fig. 1 G), as do the ascospores. These appressoria are also formed on aerial hyphae in culture and become pigmented and thick-walled like the conidia (Pl. 23, fig. 7). The two valves of the conidium do not gape apart on germination as do those of *Papularia arundinis* and *P. sphaerosperma*.

On sugar cane the conidial clusters are similar if formed on the harder woody tissues of the leaf midrib, but conidia are also produced on both leaf surfaces, as on *Arundo donax* and *Panicum maximum*, occurring in isolated groups on the surface mycelium when the leaves first become moribund. Later they appear as slit-shaped or rounded clusters erumpent through the epidermis of the leaf lamina or leaf sheath with the torn epidermis persisting as a frill.

Mason (1933) gave a list of synonyms for *Papularia vinosa*, which had been recorded on sugar cane from Antigua, Uganda and the Philippines. Other hosts recorded by Mason in Herb. I.M.I. include *Zea mays*, *Bambusa blumeana* and *Setaria chevalieri*. Penzig & Saccardo (1901) described conidial *A. camptospora* as *Melanconium saccharinum*, again from Java on dead leaves of sugar cane. They considered that the fructifications were acervuli which were hypophyllous, gregarious and longitudinally seriate, 1 mm. long × 0·5 mm. broad, black, erumpent through the epidermis, with the majority of the conidia globose, compressed, 24 × 14$\mu$, black, and borne on slender, filiform hyphae. Johnston (1917) recorded *M. saccharinum* as occurring on sugar cane in Puerto Rico, Java, Cuba and the southern United States.

Höhnel (1919) used the name *Papularia saccharina*, suggesting, but giving

no proof, that it represented the conidial state of *Apiospora camptospora*. It is of interest here to record that Ellis & Everhart stated (1892) that the dimensions of the ascospores of *Apiospora montagnei* on culms of *Arundinaria* sp. in Alabama were 20–25 × 8–10 μ, which agreed with those given by Saccardo for that species, whereas ascospores of *A. montagnei* on *Arundinaria* sp. in Louisiana measured 35–40 × 10 μ. The latter come within the range given in Table 1 for *A. camptospora*. Shortly afterwards Ellis & Everhart described *Melanconium arundinaceum* as a new species with conidia globose, 15–20 μ diam., or elliptical, 18–22 × 13–16 μ, on culms of *Arundinaria* sp. from Louisiana. This could possibly be *Papularia vinosa*.

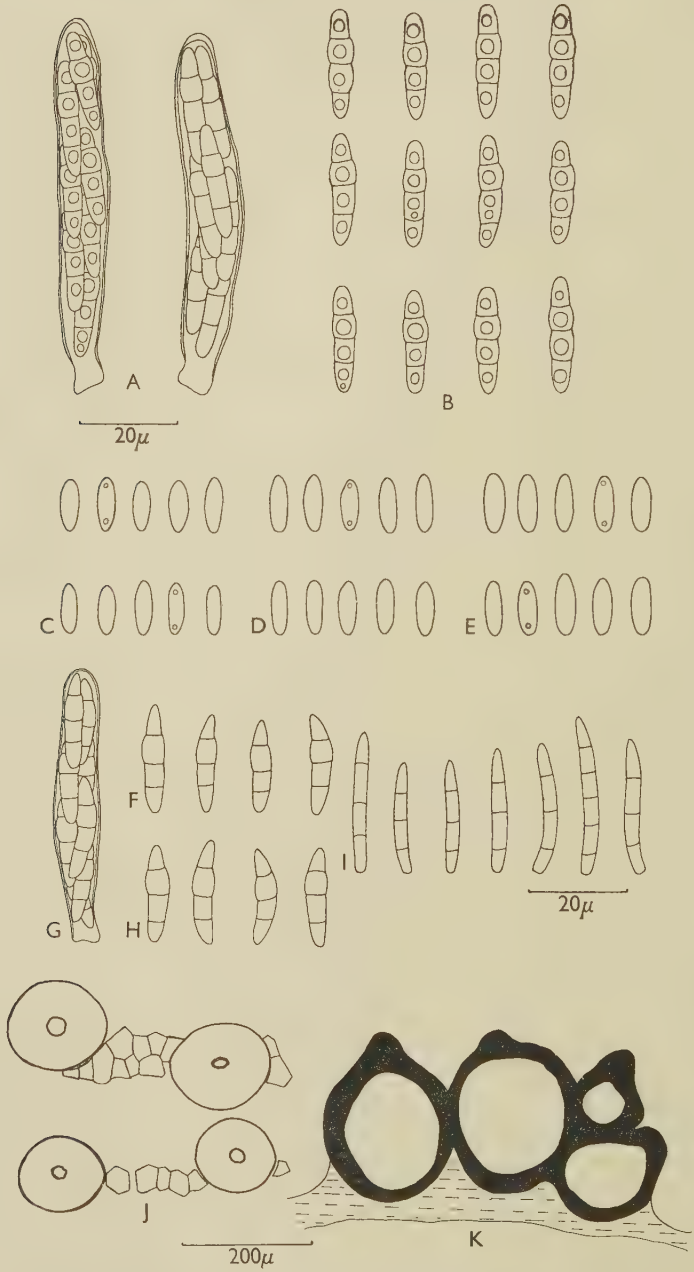
Ellis, Ellis & Ellis (1951) have shown that *Arthrinium curvatum* var. *minus* is the conidial state of a species of *Pseudoguignardia*. They figure one ascospore with a sub-median septum which is similar to that of *Apiospora*. *Papularia* and *Arthrinium* both have the diagnostic character of a flattened conidium attached to the conidiophore by one point on its periphery. Mason (1933) included these in a new tribe, the 'Bivalvae'. Ellis *et al.* (1951) point out that these two genera have other characters in common, for example the frequent formation of a superficial, close network of interlocking hyphae bearing conidiophores with a swollen, phialide-like cell at the base of each. The thick brown septum which is always found in *Arthrinium* is also found in *Papularia vinosa*. It appears also that their perfect states may be similar.

*Leptosphaeria sacchari* van Breda de Haan, *Meded. Proefstat. Suikerr. W. Java*, **3**, 25–28, 1892. (Text-fig. 2)

This fungus is said to produce ring spot of leaves of sugar cane though some authors, especially Bourne (1934), state that its parasitic role has not been adequately demonstrated. However, ring spot lesions are characteristic. They first appear as small purplish discoloured areas on the leaf surface, expand and become spherical, elongate or irregular, and may run together. As they enlarge the centres of the lesions dry and appear white, the margin being sharply demarcated from the healthy tissues. Perithecia develop within these lesions, usually in rows, and appear as black dots breaking through from beneath the epidermis.

The *perithecia* are small, globose, pale brown with a papillate ostiole raising the epidermis, and are 110–140 μ diam. *Asci* are numerous, cylindrical or club-shaped, sessile, bitunicate with rounded apex, 8-spored and measure 60–72 × 9–13 μ. *Ascospores* are irregularly biseriate in the upper part of ascus, almost hyaline, 3-septate, not or only slightly constricted at the septa and the second cell is swollen (Text-fig. 2 B; Pl. 23, fig. 1). Each cell of the ascospore has characteristically a single large guttule. The ascospores are 21–25 μ long by 4·5–6 μ wide at the swollen second cell and 3·5–5 μ elsewhere.

Single ascospore cultures later transferred to oatmeal agar produced slow-growing white colonies which soon turned pink to umber with sparse aerial mycelium. Pycnidia 180–350 μ diam. were produced all over the surface and were pinkish at first but later turned pale brown. The pycnospores were 7–13 × 3–5 μ, aseptate, individually hyaline but pale brown in




Text-fig. 2. A–E, *Leptosphaeria sacchari*. A, Asci; B, ascospores; C, pycnospores from host; D, pycnospores from a single ascospore culture; E, pycnospores from a single pycnospore culture. F–I, *L. eustomoides*. F, ascospores and G, ascus from *Saccharum officinarum*; H, ascospores from single ascospore culture; *I*, *Hendersonia* type conidia from single-ascospore culture. J–K, *L. Sacchari*. J, surface view of perithecia and pycnidia; K, section of oatmeal agar culture with pycnidia.

mass, fusoid and often with a small guttule at either end. Perithecia were not formed in culture.

Similar pycnidia occur on the host with the perithecia and are almost identical with them except that they are smaller (up to 100 $\mu$ diam.). Pycnospores from the host were 9–13 × 3–4 $\mu$. This state is referable to the form genus *Phyllosticta*. Single pycnospore cultures were identical with ascospore cultures and produced only pycnidia.

*L. sacchari* is not the only fungus associated with the early stages of ring spot lesions on sugar cane. Other fungi found by the author on typical ring spot lesions in the presence or absence of *L. sacchari* include *Helminthosporium sacchari* (van Breda de Haan) Butl., *Nigrospora sacchari* (Speg.) Mason, *Phyllosticta sorghina* Sacc., *L. eustomoides* Sacc., *Coniothyrium* sp., *Periconiella echinochloae* Batista, and numerous others. Perithecia and pycnidia of *L. sacchari* are not restricted to lesions on living leaves. The fungus persists saprophytically on old dead leaves and, though the outline of the spots can still be detected, the mycelium spreads from these, and perithecia and pycnidia are produced often in great numbers all over the leaf surface.

*L. sacchari* is a very common sugar cane leaf spot fungus. Bancroft (1910) stated that it occurred in the West Indies, Java and India and Johnston (1917) recorded it as the most prevalent leaf disease of sugar cane in British Guiana, affecting seedlings and all varieties alike. Ashby (1912) found it in Jamaica on small cultivations in the eastern parts. It appears to be common in most cane-producing countries but is of little economic importance.

Van Breda de Haan (1892) described leaf spots as 0·5–1 cm., irregularly oval, dark green at first with the centre becoming brown and later colourless on drying out and separated from healthy parts by a red-brown margin. Perithecia were formed before the spots shrivelled. They were globular, thin-walled, brown and about 140 $\mu$ diam. The ascospores were 4-celled with one thicker main cell and one or two smaller cells on either side, and were 20–24 $\mu$ long, and the breadth of the thinner end cells was at the most 3 $\mu$. They became dark in colour when fully mature and also when they had been some time outside the asci. Van Breda de Haan also described rounded red-brown conidiferous bodies, which produced at their tips smoky brown conidia consisting of three to four cells, oval and slightly curved, resembling the conidia of a *Dematium*. He was not certain of the connexion between the two states.

Since van Breda de Haan's paper most authors have agreed on the morphological symptoms of the disease, but descriptions of both the perithecial and conidial states have been varied and confused. Butler (1906) described the perithecia as sunken, only visible on the under side of the leaves, and having 8-spored asci and 4-celled ascospores, 20 × 5 $\mu$, the middle two cells broader than the rest. The probable conidial state he described as spores 20 × 9 $\mu$ formed on unbranched filaments and consisting of one large central cell with unequal sides and one or two smaller cells at the ends. His figures of ascospores are unlike those of *Leptosphaeria sacchari*. Bancroft (1910) stated that the ascospores were 3-septate, 20–24 × 5 $\mu$, and brownish, and that on the leaf surface hyphae produced 3- to 4-septate curved spores. Ashby (1912) described the asci as containing 8 4-celled

hyaline ascospores, 21–23 × 3·5–4 μ, constricted at the septa, each cell with an oil droplet and the two median cells larger. Johnston (1917) described the perithecia as spherical, thin-walled, brown and 140 μ diam.; the ascospores 3-septate, brownish, 20–24 × 5 μ; and the conidia borne on the surface hyphae. This description probably came from Bancroft (1910). Van der Bigh (1921) stated that a *Phoma* was part of the life history of *L. sacchari*. Bourne (1934) was the first to obtain a genetical connexion between *L. sacchari* and *Phyllosticta* sp. In a study of the ring spot disease of sugar cane he concluded that the pathogen was *Helminthosporium ocellum* Faris (*H. sacchari* (van Breda de Haan) Butl.) and that *L. sacchari* was an associated saprophyte. He made tissue plantings and single ascospore isolates from lesions of *L. sacchari* and obtained on glucose potato agar colonies with burnt umber edges and darker centres. These colonies produced abundant *Phyllosticta* pycnidia. Proof that the two states were genetically connected was furnished by the fact that the cultures from single ascospores and pycnospores appeared to be identical, and also because single ascospores yielded the perfect state at first on sterile sugar cane leaves and later gave only the conidial state. Unfortunately he gave neither figures nor measurements of ascospores or conidia, but a photograph of germinating conidia agrees with the Jamaican isolates. Bourne stated that the *Phyllosticta* state agrees with *P. saccharicola* P. Henn. Hennings (1907) described *P. saccharicola* from leaf spots on sugar cane leaves from the Belgian Congo. The pycnidia were hypophyllous, 60–90 μ diam. with oblong, subfusoid, hyaline, 2–3-guttulate pycnospores, 10–30 × 3–3·5 μ. The Jamaican isolates have not produced pycnospores longer than 14 μ and the pycnidia occur on both leaf surfaces.

Matsumoto & Yamamoto (1936) also found that *L. sacchari* produced abundant *Phyllosticta* pycnidia besides the perithecia on certain culture media and on natural lesions, thus substantiating the genetic connexion observed by Bourne. Luc (1953) observed on ring spot lesions very numerous *Phyllosticta* pycnidia which he described as producing fusoid spores, 9–14 × 3·5–4 μ. These measurements are in close agreement with those of the Jamaican collections. Luc stated that his spores agreed more closely with those of *Phoma* sp. described by Bouriquet in Madagascar (8·5–14·5 × 4 μ) than with *Phyllosticta sacchari*, the spores of which are longer and narrower (10–30 × 3–3·5 μ). But he was probably referring to *P. saccharicola* P. Henn. and not *P. sacchari* Speg. which has spores 4–6 × 2–3 μ. *P. sacchari* is synonymous with *P. sorghina* Sacc., which as stated above also occurs on ring spot lesions. Luc's description of the perithecial state is similar to the one given above except that his ascospores turned brown at maturity. Instead of suggesting a new combination for the conidial state, it would perhaps be better to quote Sprague (1950): 'Another species of *Phyllosticta* on sugar cane, similar to, if not identical with, *P. saccharicola* P. Henn., is the pycnidial stage of *L. sacchari* van Breda de Haan.'

Some of the discrepancies noted above in the descriptions of the perfect states may be due to the occurrence of more than one species of *Leptosphaeria* on sugar cane leaves, each having 3-septate ascospores and the second cell swollen. *L. eustomoides* is very common on both dead and dying leaves and also on ring spot lesions. It has also been

collected by the author on *Sorghum halepense*, *Arundo donax*, *Trichachne insularis* and *Melocanna bambusoides*. The ascospores could be mistaken for those of *L. sacchari*. On sugar cane the perithecia are small, black, globose, 100–160 $\mu$ diam. with numerous cylindrical, 8-spored, bitunicate asci, 44–50 × 8–9 $\mu$. The ascospores (Text-fig. 2F) are 16–22 × 4–5 $\mu$, 3-septate, only slightly constricted at the septa with the second cell enlarged, and hyaline at first but soon becoming yellowish brown. These ascospores are similar to those of *L. sacchari* except that they are narrower, yellowish brown, have more pointed end cells and do not possess the characteristic guttules (Pl. 23, figs. 1, 2). Webster & Hudson (1957) described *Hendersonia* type conidia for *L. eustomoides* on *Dactylis glomerata* and *Agropyron repens*. Similar *Hendersonia* conidia have been noted on the hosts listed above, and single ascospore isolates from sugar cane produced both perithecia and pycnidia in culture after 21 days. Mature pycnospores were 22–40 × 3–4 $\mu$, 3- to 5-septate, mostly 3-, pale brown, truncate at the base and tapering to the apex. On the host they are similar, measuring 21–29 × 2·5–3·5 $\mu$. Thus the conidial states of *L. sacchari* and *L. eustomoides* are very different. *L. spegazzinia* Sacc. & Syd. (syn. *L. sacchari* Speg.) has also been recorded on *Saccharum*. It also is similar to *L. sacchari*. It has 3-septate ascospores with a constriction at the centre and they are pale olivaceous, measuring 25 × 5–6 $\mu$, and each cell often has a central guttule.

*L. sacchari* has not been recorded on any host other than *Saccharum* sp., but Petrak & Deighton (1952) described *L. penniseticola* as a new species on *Pennisetum purpureum* where it forms lesions similar to those of *L. sacchari* on *Saccharum*.

I am grateful to Dr J. Webster for his continued advice and criticism, and to Mr E. W. Mason for his assistance with *Apiospora*.

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## EXPLANATION OF PLATE 23

Fig. 1. Ascospores of *Leptosphaeria sacchari*.
Fig. 2. Ascospores of *L. eustomoides*.
Fig. 3. *Phyllosticta* conidia of *L. sacchari* from *Saccharum officinarum*.
Figs. 4, 5. Ascospore of *Apiospora camptospora* from *Sorghum halepense*.
Fig. 6. A detached conidium of *Papularia vinosa* showing hyaline rim round the periphery.
Fig. 7. Appressorium, which has become thick-walled and pigmented, produced from a germinating conidium.
Figs. 8, 9. Attached conidia of *P. vinosa* from *Sorghum halepense* showing swollen basal cell of conidiophore. (All × 1000.)

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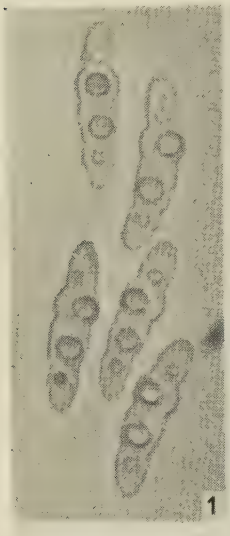


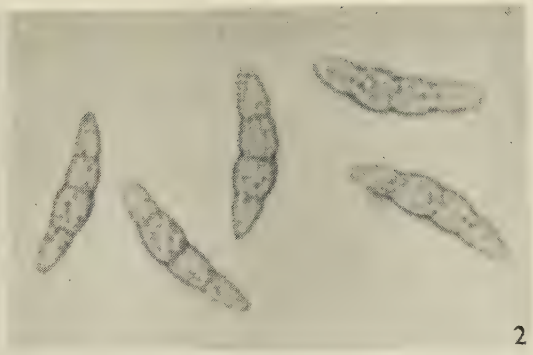

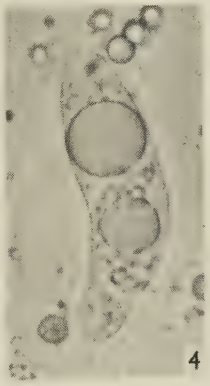

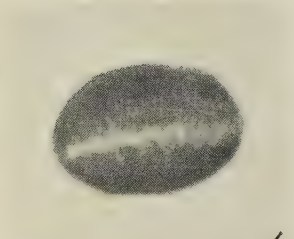


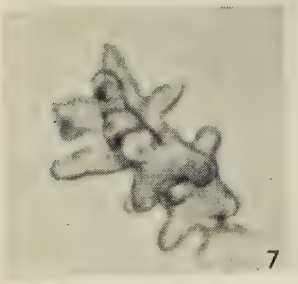

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*Trans. Brit. mycol. Soc.* **43** (4), 617–630 (1960).

# VIABLE MOULDS IN HOUSE DUST

By R. R. DAVIES

*Biological Department, St Thomas's Hospital Medical School, London**

(With 5 Text-figures)

Isolated from house dust from different parts of Britain were fifty-three species of moulds representing twenty-seven genera. If *Cladosporium* is left out of consideration and no colonization has occurred, the dust spora appears to reflect that of the out-door atmosphere.

Colonization of house dust by moulds was investigated by keeping dust samples under different conditions of atmospheric humidity. At 85% R.H. and below, *Aspergillus repens* was the dominant species, but above 85% more species developed and an ecological succession occurred.

In association with Dr Kate Maunsell it was discovered that differences in the allergenic properties of different dust samples were unrelated to their content of viable mould spores: mould and house dust allergens are distinct. Moulds in house dust utilize dust allergen(s) in their metabolism.

It has long been established that the inhalation of fungal spores, and also of house dust, can cause acute symptoms in allergic individuals. Although house dust is known to contain mould spores, their importance in dust allergy has been a subject of controversy. Brown (1936), who described moulds as the weeds of the home, Hopkins, Benham & Kesten (1930), and Flood (1931) all believed that in certain patients moulds were responsible for the dust allergy. On the other hand, Cohen, Nelson & Reinarz (1935) and Reyman & Schwartz (1947) concluded that reactions to house dust are not due to moulds. Since the differences in these views suggested a lack of precise knowledge, an investigation was initiated to determine whether or not the dust spora reflects that of the atmosphere outdoors, and to compare it with the allergenic properties of dust samples.

## Moulds isolated from house dust

In an initial study bulk dust samples were collected during the routine cleaning of private houses and hotels in different parts of Britain and a random sample from each of these was examined by the dilution plate method. Since a large amount of the material in house dust is non-wettable (including some spores) a wetting agent, 'Tween 80' at 0·02% was added to the sterile-distilled water in which the dust was to be suspended. The plates were poured at a dilution of 1:10,000 in 2% malt extract agar and first examined after 5 days' incubation.

A list of the more commonly isolated genera is given in Table 1. Specific determination was reserved for the more rarely encountered moulds (Table 2).

* Now at Commonwealth Mycological Institute, Kew.

Of the 1679 mould colonies isolated two only were of *Cladosporium*, and since most workers agree that *Cladosporium* is by far the most abundant constituent of the air spora, this result was unexpected. That the spores of this ubiquitous genus should fail to be deposited in house dust is inconceivable and it would seem more probable that in house dust *Cladosporium* spores lose their ability to germinate.

Table 1. *Number of colonies of the more common genera occurring in five Petri dishes*

| Dust samples ... | A | B | C | D | E | F | G | H | J | K | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Penicillium* | 13 | 9 | 8 | 46 | 55 | 10 | 21 | 1160 | 129 | 7 | 1458 |
| *Phoma* | . | . | . | 19 | 2 | 10 | 13 | . | . | . | 44 |
| *Mycelia sterilia* | 3 | 4 | 5 | 10 | 4 | 4 | 2 | . | . | 6 | 38 |
| *Pullularia* | 1 | 20 | 2 | 2 | 1 | 1 | 7 | . | . | 3 | 37 |
| *Mucor* | 3 | 3 | 12 | 1 | 3 | 1 | 3 | . | 3 | 1 | 30 |
| *Aspergillus* | 2 | 2 | 2 | 2 | 4 | 4 | 3 | . | 4 | 5 | 28 |
| *Stemphylium* | . | . | 3 | . | 4 | 2 | . | . | . | . | 9 |
| *White yeasts* | 35 | 18 | 15 | 10 | 14 | 40 | 50 | . | 13 | 27 | 222 |
| *Red yeasts* (+ *Sporobolomyces*) | 1 | 3 | 7 | 2 | 4 | 3 | 3 | . | 4 | . | 27 |
| *Bacteria* | 17 | 12 | 11 | 5 | 16 | 36 | 2 | . | 2 | 10 | 111 |

To obtain the number/g. dust these numbers should be multiplied by 2000.

Another unexpected feature was the isolation of the plant pathogen *Verticillium alboatrum* from dust which had been collected during the vacuum cleaning of the carpets in an hotel in Edinburgh.

If *Cladosporium* is left out of consideration the moulds most commonly isolated from the dust samples, viz. *Penicillium*, *Pullularia*, *Phoma*, *Mycelia sterilia* and *Aspergillus*, are those which Hyde & Williams (1949) and Richards (1956) found to be most frequently deposited from the air outdoors. Also, species of all the genera in Table 2 have been recorded by Richards as present in the British atmosphere.

The fact that so many *Penicillium* colonies were isolated from sample H (Table 1), however, suggested the possibility that in this sample, obtained from a damp house in urban London, colonization of the dust, followed by spore production, had occurred. It was therefore decided to investigate not only the moulds present in house dust but also which species, given suitable conditions of humidity, are able to grow in dust. This also was of allergenic interest since Wagner & Rackemann (1937) found that when pure kapock and boll cotton were inoculated with moulds and incubated, allergenic substances arose in the initially inactive material; moreover, Reyman & Schwartz (1947), Maunsell (1952) and Fior & Teseo (1955) have suggested that the constituents of house dust may be modified by the activities of fungi.

In further investigations of samples of house dust, not only from different parts of Britain but also from other countries, including India, Mauritius, Canary Isles, Switzerland and Israel, *Cladosporium* was rarely isolated.

The fungi detected in these overseas dusts were similar to those in the British samples with the addition of *Curvularia lunata* in dust from Mauritius and *C. intermedia* in dust from Israel. Table 2 is a record of the fungi isolated from the samples examined.

## The development of moulds in house dust

Though the moulds which colonize various other materials and the factors which influence their growth, particularly humidity, have been the subject of a number of reports, many of which have been summarized by Snow (1949), the development of moulds in house dust does not appear to have been studied.

Table 2. *Fungi isolated from house dust*

Phycomycetes

*Absidia corymbifera* (Cohn) Sacc.
*A. ramosa* (Lindt) Lendner
*Syncephalastrum racemosum* (Cohn) Schröter
*Mucor racemosus* Fres.
*M. spinosus* van Tieghem

Ascomycetes

*Eurotium repens* (Corda) Sacc.
*Chaetomium* spp.

Fungi Imperfecti

*Aleurisma* spp.
*Alternaria tenuis* auct.
*Aspergillus fumigatus* Fres.
*A. versicolor* (Vuill.) Tirab.
*A. sydowi* (Bain. & Sart.) Thom & Church
*A. ustus* (Bain.) Thom & Church
*A. candidus* Link
*A. niger* van Tieghem
*A. flavus* Link
*Botryotrichum atrogriseum* van Beyma
*Botrytis cinerea* Pers. ex Fr.
*Cephalosporium* sp.
*Penicillium janthinellum* Biourge
*P. palitans Westl.*
*P. nigricans* Bain.
*P. corylophilum* Dierckx
*P. citreoviride* Biourge
*P. chrysogenum* Thom
*P. brevicompactum* Dierckx
*P. roquefortii* Thom
*P. viridicatum* Westl.
*P. cyclopium* Westl.
*P. expansum* Link emend. Thom
*P. crustosum* Thom
*Cladosporium herbarum* Link ex. Fr.
*Curvularia intermedia* Boedijn
*Curvularia lunata* (Wakker) Boedijn
*Epicoccum* sp.
*Fusarium* sp.
*Gliocladium roseum* (Link) Bain.
*Gliomastix convoluta* (Harz) Mason
*Papularia sphaerosperma* (Pers. ex Fr.) Hoehnel
*Papulospora coprophila* (Zukal) Hotson
*Penicillium thomii* Maire
*P. frequentans* Westl.
*P. lilacinum* Thom.
*P. purpurogenum* Stoll
*P. variabile* Sopp
*P. piceum* Raper & Fennel
*Phoma* sp.
*Pullularia pullulans* (De Bary & Low) Berkh.
*Scopulariopsis brevicaulis* (Sacc.) Bain.
*Stachybotrys atra* Corda ex Fr.
*Stemphylium lanuginosum* Harz
*Trichoderma viride* Pers. ex Fr.
*Tricothecium roseum* Link ex Fr.
*Verticillium alboatrum* Reinke & Berth.
*V. nigrescens* Pethyb.

### *Experimental methods*

To determine which moulds colonize house dust and the relationship between colonization and atmospheric humidity dust damples were incubated in glass jars (4·5 l. capacity) with screw lids at controlled humidity, the required water vapour pressures being obtained by means of sulphuric acid solutions (Wilson, 1921; Solomon, 1951). Each jar contained 500 ml. of acid and a small stand to support the dish of dust.

The bulk dust samples used were A and B, collected during the routine vacuum cleaning of a surburban house in North London, and C from the top of a four-poster bed in a farmhouse in Dorset. After thoroughly mixing a sample randomly selected weighed portions were placed in sterile Petri-dish lids, each was teased out with sterile needles so that it

covered the surface of the lid as a relatively uniform mat, and the lid was then placed in the jar.

R.H. employed ranged from 60 to 97·8 % (Figs. 1–5). The chambers for sample A were kept in the dark in an incubator at 25° C., and since there is very little change in R.H. with variations in temperature of up to ± 5° C., those with samples B and C were kept on the laboratory bench (av. 19° C.) where temperature fluctuations were smaller than this. Portions of B and C not in humidity chambers were kept on the laboratory bench. It may be assumed that these were in moisture equilibrium with the air of the laboratory, which during the period of the experiments varied between 45 and 55 % R.H.

At regular intervals, without removal from the chambers, the dust samples were examined with the unaided eye, and at 14-day intervals under the microscope, and when sporophores were seen small fragments of the dust associated with them were removed to nutrient agar. A representative collection of the moulds was thus obtained.

At the end of the incubation periods the number of spores per g. dust was taken as a measure of mould development. For A this was estimated by means of a direct haemocytometer count of the number of spores in suspensions of 'mouldy' dust in 'Tween 80'. For B and C, using 9 cm. Petri dishes, dilution plates (ranging from 1:10,000 to 1:50,000,000) were poured in 2 % malt extract agar.

## Observations

### *Sample A*

After 14 days a conspicuous overgrowth of *Absidia corymbifera* and *Mucor racemosus* had developed in the dust at R.H. 97·8 and 93·7 %. At lower R.H. no mycelium was visible to the unaided eye, but under the microscope hyphae were seen in samples at 88·8 and 84·4 % R.H.

By the 28th day most of the mucoraceous overgrowth on the dust at 97·8 and 93·7 % R.H. had collapsed and patches of dense white subaerial mycelium of *Penicillium chrysogenum* were evident. Under the microscope a profusion of hyphae could be seen, and conidiophores of *Penicillium*, *Aspergillus*, *Tricothecium*, *Gliocladium*, *Scopulariopsis*, *Fusarium* and *Cephalosporium* were identified. In some instances *S. brevicaulis* and *A. versicolor* appeared to be growing on fragments of hair. In the dust at the lower R.H. no mycelium was visible to the unaided eye at this stage. However, under the microscope *Penicillium* conidiophores were visible in the dust at 88·8 % R.H., but in that at 84·4 % R.H., though hyphal growth had occurred, no conidiophores were seen.

Between the 4th and 6th week, at 97·8 and 93·7 % R.H. the patches of dense white subaerial mycelium became more conspicuous and *P. chrysogenum* appeared to be the dominant fungus. After 6 weeks at 97·8 and 93·7 % R.H., the conidiophores of *Gliomastix convoluta* were detected under the microscope. With the passage of time, this mould appeared increasingly to flourish, until at the end of 12 weeks it had overgrown the mats of mycelia and dust in the 97·8 and 93·7 % R.H. chambers. As it became dominant the mats changed from greyish white to dirty grey.

Between the 6th and 12th week the conidiophores of *Penicillium* and *Aspergillus* were identified in the 88·8 % dust, but only vegetative hyphae were observed in that at 84·4 and 80·5 % R.H.

The number of spores in each sample at the end of the 12th week, estimated by a haemocytometer count, are given in Fig. 1. Other moulds which colonized dust at 93·7 and 97·8 % R.H. were *P. brevicompactum*, *P. crustosum*, *P. variabile*, *Aspergillus glaucus* (*Eurotium repens*)*, *Gliocladium roseum*, *Tricothecium roseum*, *Cephalosporium* (hyaline) spp., and *Fusarium* spp.

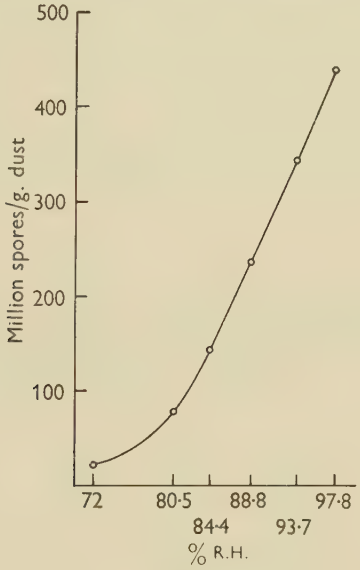


Fig. 1. Number of spores per g., in sample A house dust stored for 84 days at different humidities.

*Sample B*

After 3 weeks mould development, visible to the unaided eye in the dust at 95 and 90 % R.H. only, appeared as a greyish white mat of mycelium with tiny spots of dense white and green mildew. In 95 % R.H. dust conidiophores of *Aspergillus*, *Penicillium*, *Scopulariopsis*, *Cephalosporium*, *Aleurisma*, *Tricothecium* and *Papularia* and bright yellow and orange cleistothecia were visible. There appeared to be three species of *Aspergillus*: one with large white heads, probably *A. candidus*, another with smaller green columnar heads, probably *A. versicolor*, while the third with large pale yellow heads was probably *Eurotium repens*. These three species were frequently identified later in a large number of isolates studied in culture.

In the 90 % R.H. dust the conidiophores of the same three species and at least two species of *Penicillium* were observed, but though *Scopulariopsis* was present it was much less in evidence than in the others. The small orange and yellow cleistothecia were again evident. At this stage mycelial

* All the colonies of the *Aspergillus glaucus* series did not produce cleistothecia, but those which did were identified as *Eurotium repens*.

growth could be seen under the microscope in the dust at 85 and at 80 % R.H. but not in that at 75 % and below.

After 6 weeks the 95 % R.H. dust samples showed a profuse development of loosely weft, white subaerial and aerial mycelium, with patches of very dense white mycelium. Subsequently, *Mucor racemosus*, *M. spinosus*, *P. brevicompactum*, *P. frequentans* and a *Fusarium* sp. were identified in cultures from this mycelial overgrowth. From the dense white type *P. brevicompactum*, *P. frequentans*, *A. candidus* and *S. brevicaulis* were isolated. Preparations of this growth in lactophenol blue contained *Aleurisma* sp., but it was not isolated. *Aspergillus*, *Scopulariopsis*, *Tricothecium* and the orange and yellow cleistothecia were conspicuous under the microscope.

After 6 weeks the 90 % R.H. dust was markedly mouldy to the unaided eye, bearing a loose mat of subaerial and aerial mycelium and small but conspicuous dense white spots, which proved to contain *A. candidus* and *A. versicolor*. From a microscopic examination *Aspergillus* appeared to be dominant, though both *Penicillium*, *Scopulariopsis* and the yellow cleistothecia were present.

Table 3. *Estimated numbers of the more common mould colonies isolated in thousands/g. dust, from portions of sample B after* 6 *weeks at different humidities*

| | % relative humidity | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 95 | 90 | 85 | 80 | 75 | 70 | 65 | Control |
| *Penicillium frequentans* | 35,000 | 15,000 | 900 | . | . | 5 | 4 | 10 |
| *P. brevicompactum* | 80,000 | 25,000 | 900 | . | 3 | . | . | 6 |
| *P. cyclopium* | 25,000 | 5,000 | 300 | . | 3 | . | . | 1 |
| *P. viridicatum* | 85,000 | . | . | . | 1 | . | . | 2 |
| *P. chrysogenum* | 30,000 | 5,000 | . | . | 1 | . | . | . |
| *P. piceum* | 5,000 | 10,000 | 100 | . | . | . | . | . |
| Unidentified *P.* spp. | 5,000 | 10,000 | 100 | 100 | 1 | 1 | . | 1 |
| *Aspergillus versicolor* | 90,000 | 260,000 | 100 | 100 | 1 | 1 | . | 3 |
| *Eurotium repens* | 10,000 | 25,000 | 8,100 | 4,900 | 2 | . | . | . |
| *Aspergillus candidus* | 30,000 | 75,000 | 100 | . | . | . | 1 | . |
| *Scopulariopsis brevicaulis* | 10,000 | . | . | . | 5 | 7 | 6 | 1 |
| *Mycelia sterilia* | 175,000 | 20,000 | . | 800 | 12 | 11 | . | . |

The 85 % R.H. dust, after 6 weeks, also appeared conspicuously mouldy to the unaided eye, the dust being overgrown by a loose mat of white subaerial mycelium. It contained the same moulds and cleistothecia as were observed in the 90 % R.H. dust.

The dust at 80 % R.H. was by this time also mouldy, but mycelial development was much more sparse than at higher humidities, and microscopic examination revealed only the conidiophores of *A. glaucus*. *Eurotium repens* was later isolated.

The 75 % R.H. sample showed no mould growth to the unaided eye, though a few spore heads of *A. glaucus* were seen under the microscope.

No mould growth was distinguished in any of the other samples kept at relative humidities of less than 75 %.

At the end of the 42-day period, the lots were removed from their humid chambers, and the Petri dishes containing them covered with sterile lids. The dishes were kept on the laboratory bench to bring them into

moisture equilibrium with the air of the laboratory. This ensured that when lots were weighed for assay each contained approximately the same weight of water. The amount of mould development was determined by means of dilution plates (Figs. 2, 3; Table 3).

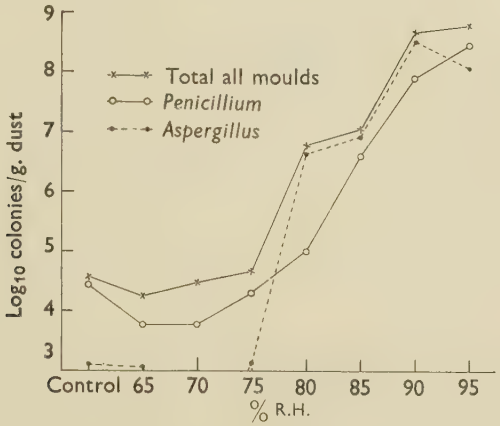


Fig. 2. Relative importance of *Aspergillus* and *Penicillium* in the mould colonization of house dust after 42 days at different humidities.

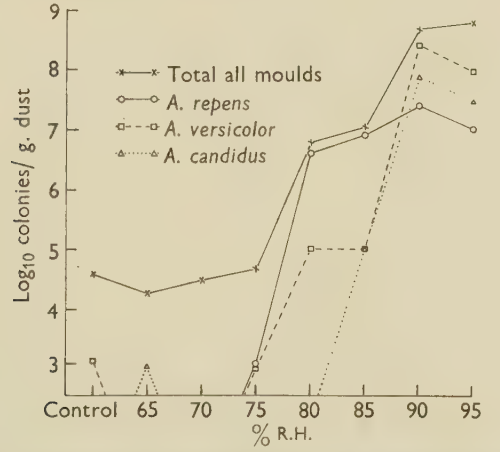


Fig. 3. Relative importance of *Aspergillus* spp. in the colonization of house dust after 42 days at different humidities.

### *Sample C*

After 14 days a thin mycelial growth was visible to the unaided eye in the dust at 95 and 90 % R.H. but not in those at lower humidities. *Penicillium*, *Aspergillus*, *Scopulariopsis* and *Cephalosporium*, and the bright yellow cleistothecia of *Eurotium repens* were identified. Mycelium and *Aspergillus* conidiophores were observed in the dust at 85 and 80 % R.H., but not in that at lower humidities.

After 28 days the lots were brought into moisture equilibrium with an atmosphere of 55 % R.H. and then examined by dilution plates (Table 4; Figs. 4, 5).

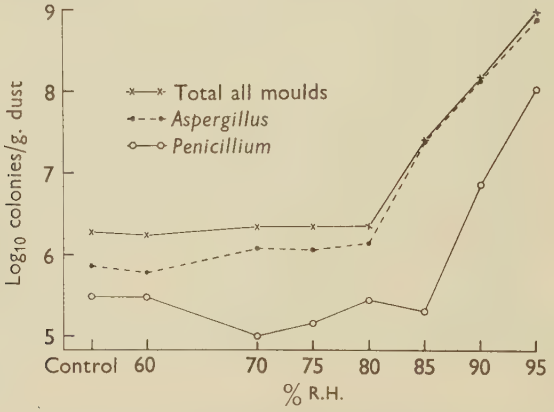


Fig. 4. Relative importance of *Aspergillus* and *Penicillium* in the colonization of house dust, after 28 days at different humidities.

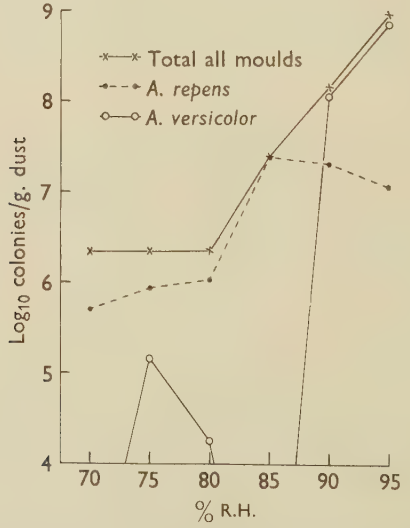


Fig. 5. Relative importance of *Aspergillus repens* and *A. versicolor* in the colonization of house dust after 28 days at different humidities.

## Discussion and conclusions

In this study the number of spores/g. dust was taken as a measure of mould development in dust at different humidities. Barton-Wright & Tomkins (1940) and Snow, Crighton & Wright (1944), however, in their studies of mould spoilage in flour and animal feeding stuffs expressed the rate of mould development on samples stored at different humidities as the reciprocal of the time taken before the appearance of mycelium. This

Table 4. *Numbers of mould colonies isolated from house dust sample C after 28 days at different humidities*

| R.H. ... | 95% | | 90% | 85% | 80% | | 75% | 70% | 60% | Control |
|---|---|---|---|---|---|---|---|---|---|---|
| | Lot 1 | Lot 2 | | | Lot 1 | Lot 2 | | | | |
| No. of plates ... | 10 | 10 | 9 | 10 | 8 | 10 | 7 | 10 | 10 | 10 |
| Dilution ... | 1:50 m. | 1:50 m. | 1:1 m. | 1:1 m. | 1:1 m. | 1:1 m. | 1:1 m. | 1:1 m. | 1:1 m. | 1:1 m. |
| *Penicillium janthinellum* | 5 | . | 1 | . | . | . | . | 1 | 1 | 1 |
| *P. nigricans* | . | . | 3 | . | . | . | . | . | . | . |
| *P. chrysogenum* | 2 | 6 | 7 | 1 | . | . | . | . | . | . |
| *P. brevicompactum* | 2 | 9 | 15 | 1 | . | 3 | . | . | . | . |
| *P. roquefortii* | . | . | 4 | . | . | . | . | . | . | . |
| *P. viridicatum* | 6 | . | 2 | . | . | . | 1 | . | . | . |
| *P. cyclopium* | 10 | . | 4 | . | 1 | . | . | . | 2 | 2 |
| *P.* spp. not identified | . | 2 | 28 | . | . | 1 | . | . | . | . |
| Total *Penicillium* | 25 | 17 | 64 | 2 | 1 | 4 | 1 | 1 | 3 | 3 |
| *Eurotium repens* | . | 2 | 183 | 243 | 6 | 13 | 6 | 5 | . | . |
| *Aspergillus versicolor* | . | 129 | 1068 | . | 1 | . | 1 | . | . | . |
| *A. flavus* | . | 1 | . | . | 1 | 3 | . | 7 | . | . |
| *A. fumigatus* | . | . | . | . | 1 | . | 1 | . | . | . |
| Total *Aspergillus* | 167 | 132 | 1251 | 243 | 9 | 16 | 8 | 12 | . | . |
| *Gliomastix convoluta* | 8 | 6 | 7 | 1 | . | . | . | . | . | . |
| *Scopulariopsis brevicaulis* | 1 | . | . | . | . | . | . | . | 2 | 2 |
| *Cephalosporium* spp. | 1 | 1 | . | . | 1 | . | 1 | . | 4 | 1 |
| *Aleurisma* spp. | . | 1 | . | . | 2 | . | 2 | . | . | . |
| *Mycelia sterilia* | 7 | 2 | . | . | 2 | 2 | 3 | 3 | 2 | 2 |
| *Pullularia pullulans* | . | . | . | . | . | . | . | 1 | . | . |
| *Chaetomium* spp. | . | . | . | . | 1 | . | . | . | . | . |
| *Cladosporium herbarum* | . | . | 2 | . | . | . | . | . | . | . |

Table 5. *The lowest humidity at which sporulation occurred in moulds colonizing house dust*

| | | Dust sample | | |
|---|---|---|---|---|
| | % R.H. | A | B | C |
| PHYCOMYCETES | | | | |
| *Absidia corymbifera* | 93·7 | + | . | . |
| *Mucor racemosus* | 93·7 | + | + | . |
| ASCOMYCETES | | | | |
| *Eurotium repens* | 75 | + | + | + |
| FUNGI IMPERFECTI | | | | |
| *Aspergillus versicolor* | 85 | + | + | + |
| *A. candidus* | 85 | . | + | . |
| *Penicillium frequentans* | 85 | . | + | . |
| *P. brevicompactum* | 85 | . | + | . |
| *P. cyclopium* | 85 | . | + | + |
| *P. chrysogenum* | 85 | + | + | + |
| *P. piceum* | 85 | . | + | + |
| *P. viridicatum* | 90 | . | + | + |
| *P. variabile* | 93·7 | + | . | . |
| *P. crustosum* | 93·7 | + | . | . |
| *Scopulariopsis brevicaulis* | 85 | + | + | + |
| *Gliomastix convoluta* | 90 | + | . | + |
| *Gliocladium roseum* | 93·7 | + | . | + |
| *Tricothecium roseum* | 93·7 | + | . | + |
| *Fusarium* sp. | 93·7 | + | . | . |
| *Papularia sphaerosperma* | 95 | + | . | + |
| *Aleurisma* sp. | 95 | + | + | . |
| *Cephalosporium* sp. | 95 | + | . | . |

+ = samples in which positive identification of colonization was obtained.

reciprocal is not only determined by humidity, but is to some extent affected by the relative rates of mycelial development of different moulds. Humidity not only affects growth and sporulation, but also determines which mould species develop, and though at very high humidities mucoraceous moulds with rapidly spreading mycelia may predominate, at lower humidities the field is left to the slower growing Aspergillaceae. In different moulds the relationship between spore production and total fungal growth may be very different, and at low humidity mycelial growth may occur without spore production.

The results illustrated in Fig. 1 show that the relationship between R.H. and mould growth in house dust is linear between 97·8 and 80 % and then curves, which, despite the experimental differences described, is comparable with the results of Barton-Wright & Tomkins (1940) and Snow *et al.* (1944).

In this study it was assumed that a mould colonized house dust if at least one of the following criteria was satisfied:

(1) Its sporophores were observed in the dust.

(2) Its colonies developed from fragments of mycelium removed from the dust and subcultured on agar.

(3) It was present in dilution plates in significantly larger numbers than those obtained from the control or from samples kept at lower humidities.

Since the species can rarely be identified from the appearance of sporophores alone, and it is frequently necessary to know colony characteristics all these criteria, though desirable, could not be satisfied.

From the observations described, and which are summarized in Table 5, it may be concluded that certain moulds, given suitable conditions of humidity, readily colonize house dust. At high R.H. (97·8, 95 and 93·7 %), colonization appears to occur in a definite succession. When present, quickly growing *Mucor* and *Absidia* species appeared first and were dominant at the end of 2 weeks. By the end of the first month *Penicillium* species assumed dominance, while in sample A, *Gliomastix convoluta* progressively increased in importance to become dominant by the end of the third month. This pattern of succession does not occur in dust at lower humidities because there is no colonization by the quickly growing mucoraceous moulds and only some species of *Penicillium* and *Aspergillus* develop.

At the lower humidities atmospheric humidity is a limiting factor and determines which genera and species develop. For instance the only conidiophores observed in dust at R.H. as low as 75 % were of the *A. glaucus* series, and *Eurotium repens* was isolated in greater numbers than any other mould from the 80 and 85 % samples (Tables 3, 4; Figs. 3, 5). The reports of such authorities as Thom & Le Fevre (1921), Bunting (1930), Koehler (1938) and Snow *et al.* (1944) that members of the *A. glaucus* group are the first moulds to appear on stored products as moisture content rises are thus confirmed. At the higher humidities, however, other species become dominant, and at 95 % R.H. the number of colonies of *E. repens* isolated was smaller than that obtained from the dust at 85 % R.H. Since it was found by experiment that when *E. repens* develops on sterilized house dust as the sole colonizer maximum development is at the highest

humidity, it is reasonable to suggest that the decrease in the numbers of colonies of *E. repens* isolated at 95 % R.H. was due to competition with the other moulds. In sample C this trend was also shown by *A. versicolor* and *A. candidus*.

At low humidities where *E. repens* is the sole colonizer competition is intra-specific, but as humidity rises to the optimum necessary for the development of other species this is added to, and intensified by, increasing inter-specific competition.

Although Hyde & Williams (1949) and Richards (1956) record *Cladosporium*, *Pullularia* and *Phoma* as being commonly deposited in Petri dishes of nutrient media exposed to the outdoor atmosphere, among the 2764 colonies examined in the dilution plates from the dust samples kept at different humidities, six only were *Cladosporium*, eighteen *Pullularia* and twenty *Phoma*, and their sporophores were never observed in any of the colonized dust mats. Since *Cladosporium* spores have been shown to germinate at 90 % R.H. by Galloway (1935) and by Chowdhury (1937), who in addition reported that 6·7 % germinated at that humidity within 70 hr., while Snow (1949) reported germination after 7 days on nutrient gelatin at 88·8 % R.H., the failure of *Cladosporium* to develop in house dust at humidities as high as 97·8 % is unlikely to be due to the lack of available water in the subsaturated atmosphere. *Cladosporium* was so rarely isolated in the initial study that it was concluded that in house dust there is a rapid loss in the ability of the spores to germinate. On the other hand *Pullularia* and *Phoma* were isolated from house dust samples which had been stored a number of weeks. Given the reasonable assumption that their viable spores were present it appears that neither *Pullularia* nor *Phoma* were able to colonize house dust in competition with other moulds even at R.H. as high as 97·8 %.

In sample C, though conidiophores of *Gliomastix* were not seen, since the number of colonies isolated indicated increasing development with increase in humidity from 85 to 95 % R.H. (Table 4), it must be concluded that colonization occurred at 95, 90 and possibly even at 85 % R.H. In this instance the dilution plate method showed evidence of mould colonization which was not observed under the microscope. On the other hand, the species of *Absidia*, *Aleurisma*, *Fusarium*, *Tricothecium* and *Papularia*, which were observed in the colonized dust mats, were not isolated in the dilution plates. The conidiophores of *Scopulariopsis* and *Cephalosporium* were observed in dust at 95 and 90 % R.H. and either not isolated or isolated in very small numbers, so that here the dilution plate method gave little or no evidence of colonization by these moulds. In addition, *M. racemosus*, *M. spinosus* and *Fusarium* sp., which were isolated when portions of mycelial overgrowth were transferred to agar media, were also absent from the dilution plates. These discrepancies may be to some extent associated with the availability of water in the respective environments presented for colonization by house dust in moisture equilibrium with a subsaturated atmosphere and those of artificial culture.

In the competitive colonization of a freshly poured nutrient gel, the soluble waste metabolites produced by the more rapidly developing colonies freely diffuse through the gel. Such staling products have

antagonistic effects and may inhibit spore germination, where it had been delayed, and possibly even induce sterility in slowly developing colonies. Sterile colonies were frequently obtained and some, when grown in pure culture on the same medium as they were originally isolated, were found to be *Scopulariopsis brevicaulis*.

In the competitive colonization of house dust in moisture equilibrium with a subsaturated atmosphere, where the amount of water available is strictly regulated by a solution of sulphuric acid, there can be no appreciable diffusion of water-soluble metabolites. The antagonistic effects resulting from the mutual development of a number of mould species will therefore be greatly diminished, and it must follow that competition between colonizing moulds in house dust is less severe than that experienced by the same species developing in an agar medium in a Petri dish.

Since Tomkins (1929), Groom & Panisset (1933) and Snow *et al.* (1944) reported that the availability of an external source of nutrients affects the range of humidity over which mould development occurs, the nutrient content of house dust is of interest.

In a chemical analysis of dust samples obtained from suburban London, a town in south-east England and Bombay, Davies (1960) found that the total weight of organic matter is a little less than a half, the fat content (ether extract) varies between approx. 3·5 and 6 %, carbohydrate between 14 and 25 %, and protein (N × 6·25) between 18 and 22 %. Most of the N occurs as scleroprotein in desquamated skin scales and hair. Cold water extracts of these dusts were found to contain from 0·1 to 0·3 % N but only a trace of reducing sugar before acid hydrolysis. After hydrolysis with $H_2SO_4$, however, values of between 2 and 6 % hexose were obtained and it was concluded that most of the soluble carbohydrate occurs as polysaccharide. From the evidence of Rimmington, Stilwell & Maunsell (1947) on the constitution of the house dust allergen (the aqueous extract), it seems probable that most of this polysaccharide is of galactose residues, and that some of the soluble N is present in such compounds as mucoproteins and mucopolysaccharides.

The utilization of the scleroprotein in house dust by most of the moulds which develop seems unlikely, since Reese & Downing (1951) found *Eurotium repens* and *A. versicolor* inactive on wool, and White, Mandels & Siu (1950) reported that *Gliomastix convoluta* and certain species of *Fusarium* and *Penicillium* attack wool only after it has been autoclaved. However, house dust contains more soluble N and carbohydrate than many artificial media, and aqueous extracts kept under non-sterile conditions invariably develop pellicles of bacteria and common fungi. Furthermore, when normal and fat-free dust samples were inoculated with dried conidia of *E. repens* and incubated at 85 % R.H., far greater numbers of colonies were isolated from normal than from defatted dust.

The metabolism which enables moulds to develop in subsaturated atmospheres does not appear to have been studied. Groom & Panisset (1933) suggested that the water produced by respiration might increase the humidity of the air in a more or less confined space, such as between the books in a library where the temperature of the room air fluctuates, but considered that this played no part in facilitating growth on materials

exposed in an enclosed atmosphere where humidity was regulated by a salt solution. It seems worthy of note, however, that food storage in fungi is largely in the form of oil droplets, and that when fat is completely oxidized rather more than an equal weight of water is produced.

## Mould and house-dust allergens

The clinical studies involved were undertaken by Dr Kate Maunsell and will be described in detail elsewhere. Extracts of the moulds isolated from house dust were compared with extracts of dust samples by means of intradermal skin tests and by determing the threshold titre (Maunsell, 1956), i.e. the reciprocal of the lowest dilution of the extract to give positive skin reactions in a group of patients.

The allergenic potencies of the dust samples differed considerably. The strongest, sample H, from a private house in urban London, contained over 2 million spores/g. of *Penicillium frequentans*, which suggested that allergenic potency might be related to the content of *Penicillium*. However, though many patients gave positive skin reactions to an extract of this dust, reactions to *P. frequentans* were rare, and no correlation was found between mould content and allergenic potency in the different dust samples.

Extracts from the dust samples colonized at 97, 95 and 90 % R.H., each of which contained more than 400 million spores/g., were no stronger than the extracts from their respective control portions containing less than 1 million spores/g. It must be concluded, therefore, that in house-dust allergenic potency is unrelated to the numbers of mould spores it contains. The contention of Cohen *et al.* (1935) and Reyman & Schwartz (1947) that house-dust allergy is not due to fungal allergens is thus supported.

Extracts from the portions of dust sample B colonized at 95 and 90 % R.H. were significantly weaker than extract from the uncolonized dust. This and the fact that bacteria and fungi invariably develop in extracts of house dust exposed to air suggested the possibility that the fungi might utilize dust allergen(s) in their metabolism. To investigate this portions of sterile extracts of house dust inoculated with different moulds and uninoculated portions were incubated. At intervals their skin reactive properties were compared in a group of dust-sensitive patients who were not allergic to mould allergens. As the fungi developed, a diminution in the allergenic activity of the dust extracts occurred. It must be concluded, therefore, that during their development moulds utilize house-dust allergen(s), and that mould and house-dust allergens are distinct. The report of Wagner & Rackemann (1937) that the mould colonization of allergenically inactive pure kapok and boll cotton led to the development of allergenic substances is most probably explained by the development of mould allergens to which their patients reacted.

I wish to express my thanks to Prof. C. T. Ingold for his kind encouragement and help throughout the course of the work, and to Dr Kate Maunsell who stimulated my interest in allergy and as clinical collaborator made the allergenic investigations possible. I am grateful to Mr George Smith for his taxonomic assistance in either confirming or identifying a number of mould species, Dr A. G. Hamilton for help in securing research facilities,

and Mr A. G. Dennise for technical assistance. The work presented forms part of a thesis accepted for the Ph.D. Degree of the University of London.

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# THE MORPHOLOGY OF *CHAETOSPERMUM CHAETOSPORUM*

By R. N. de FONSEKA
*Birkbeck College, University of London*

(With Plate 24 and 3 Text-figures)

*Chaetospermum chaetosporum* (Pat.) A. L. Smith & Ramsb. which developed on a submerged decaying leaf of alder (*Alnus*) has been grown in pure culture. The reproductive bodies are completely closed pycnidia. The development of the spore and its appendages is described and illustrated.

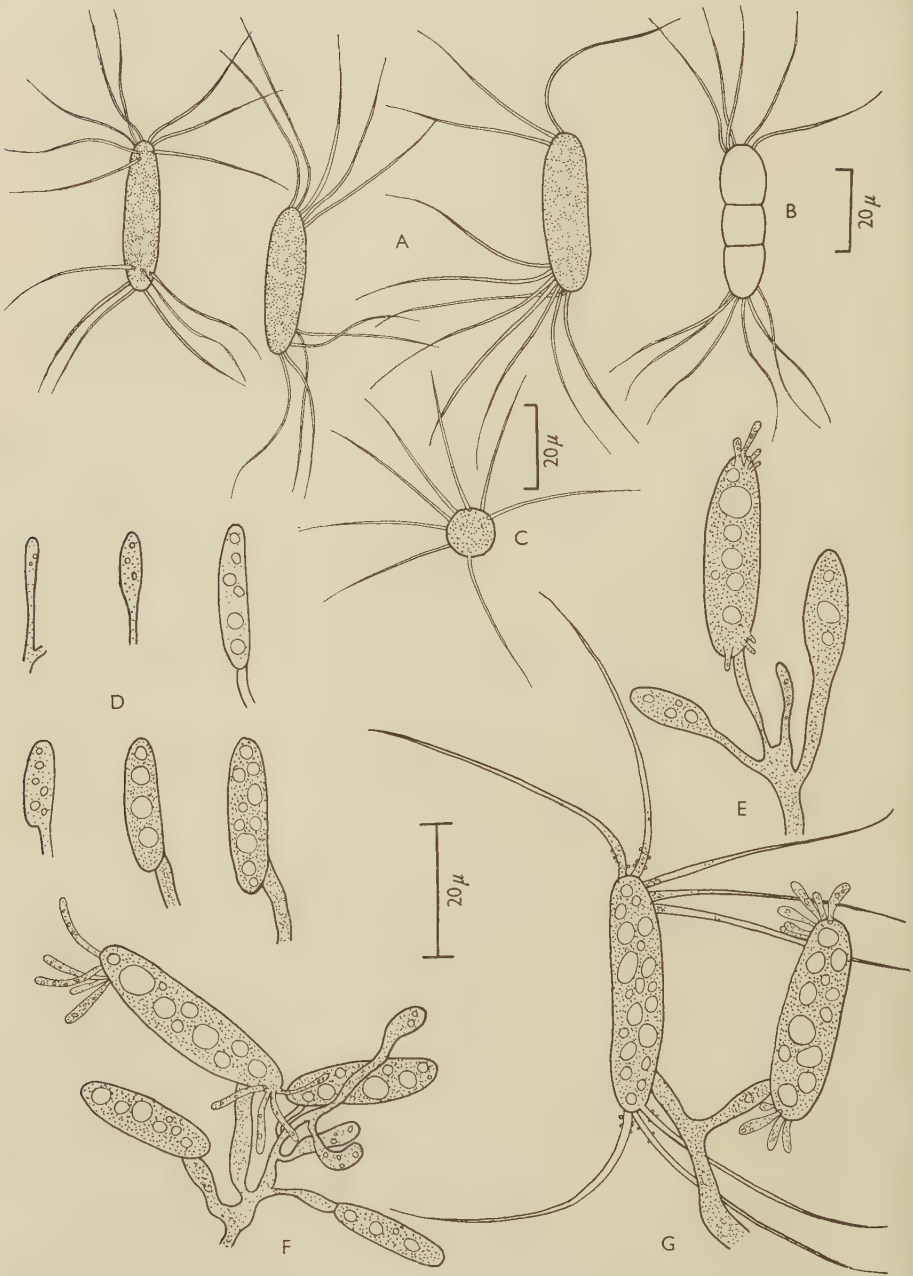
Patouillard (1888) found a fungus on decaying grass and described it as *Tubercularia chaetospora* though it differed considerably from other species of the genus. Saccardo (1892) erected a new genus, *Chaetospermum*, for Patouillard's fungus and named it *C. tuberculariodes*. Smith & Ramsbottom (1913), recording the species for Britain, reverted to the epithet *chaetosporum* in accordance with the International rules.

I observed this species in the autumn of 1958 on an alder leaf collected from the bottom of a slow moving stream flowing into Virginia Water, Surrey. In a search for aero-aquatic fungi (Glen-Bott, 1951) leaves were collected from the stream, rinsed in water and kept moist in Petri dishes at 20° C. in the light. After 3 weeks white glistening bodies appeared on an alder leaf, being particularly abundant on the midrib. From this a single-spore culture was obtained. On malt agar and on quite a wide variety of other standard media vigorous growth occurred, but no true fruiting structures were formed, though small localized tufts of aerial hyphae were sometimes produced which superficially resembled the pycnidia.*

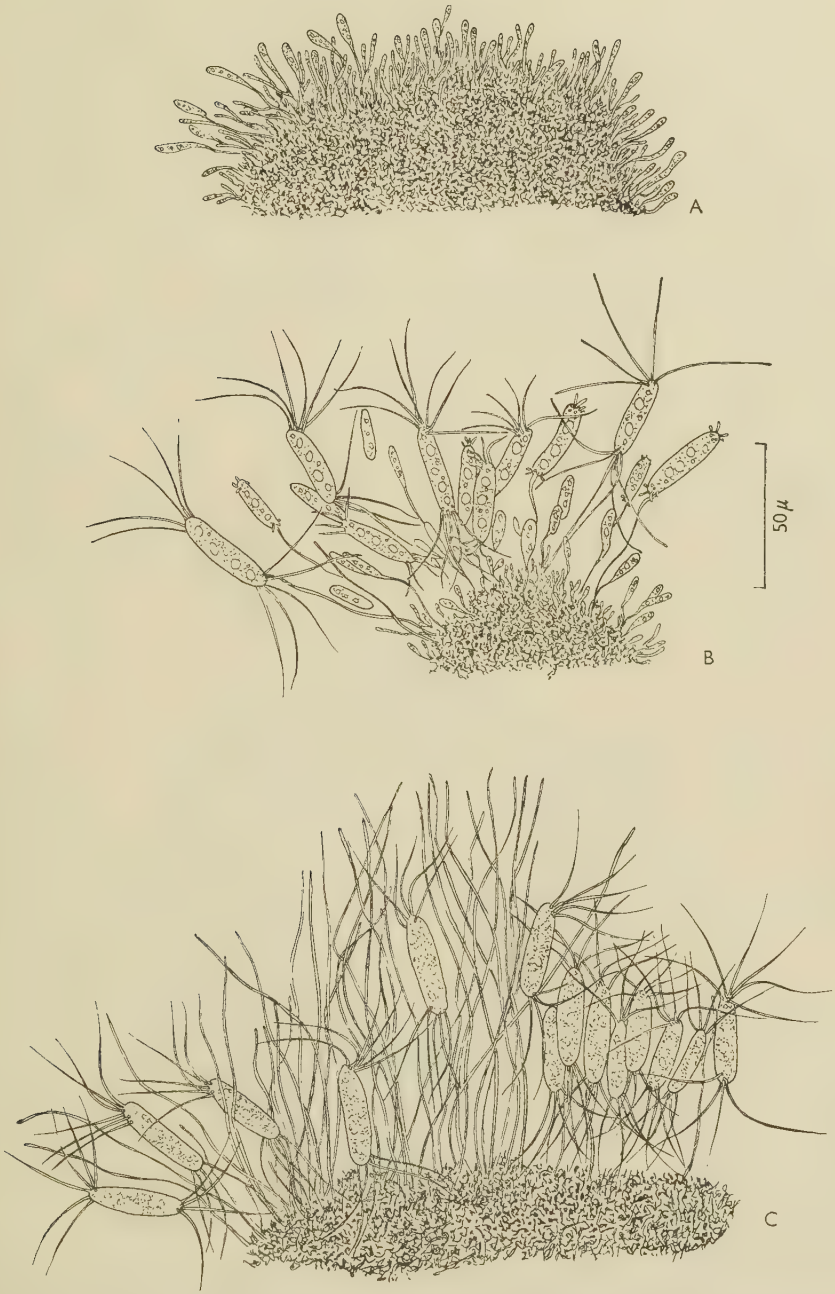
In an attempt to induce sporulation strips of alder leaf were included in the malt agar. On a medium of this kind sporulation occurs readily, the reproductive bodies appearing mainly on the leaf tissue. Furthermore, the presence of some free water in the culture vessel was found to accelerate and increase sporulation. To this end either the plates or tubes were cooled in such a way as to encourage condensation, or some drops of sterile water were added after a few days' growth had occurred.

The reproductive structures are sessile, white, superficial, entirely closed pycnidia and 0·4–2 mm. diam., occurring singly or in clusters (Pl. 24, fig. 1). Though most are roughly spherical, a few are irregular. The gregarious habit may be due to separate pycnidia arising very close to each other or to new pycnidia growing out from those already formed. One such mass had as many as 45 pycnidia. The wall of the pycnidium is 50–160 $\mu$ thick and the surface is gelatinous and white, becoming

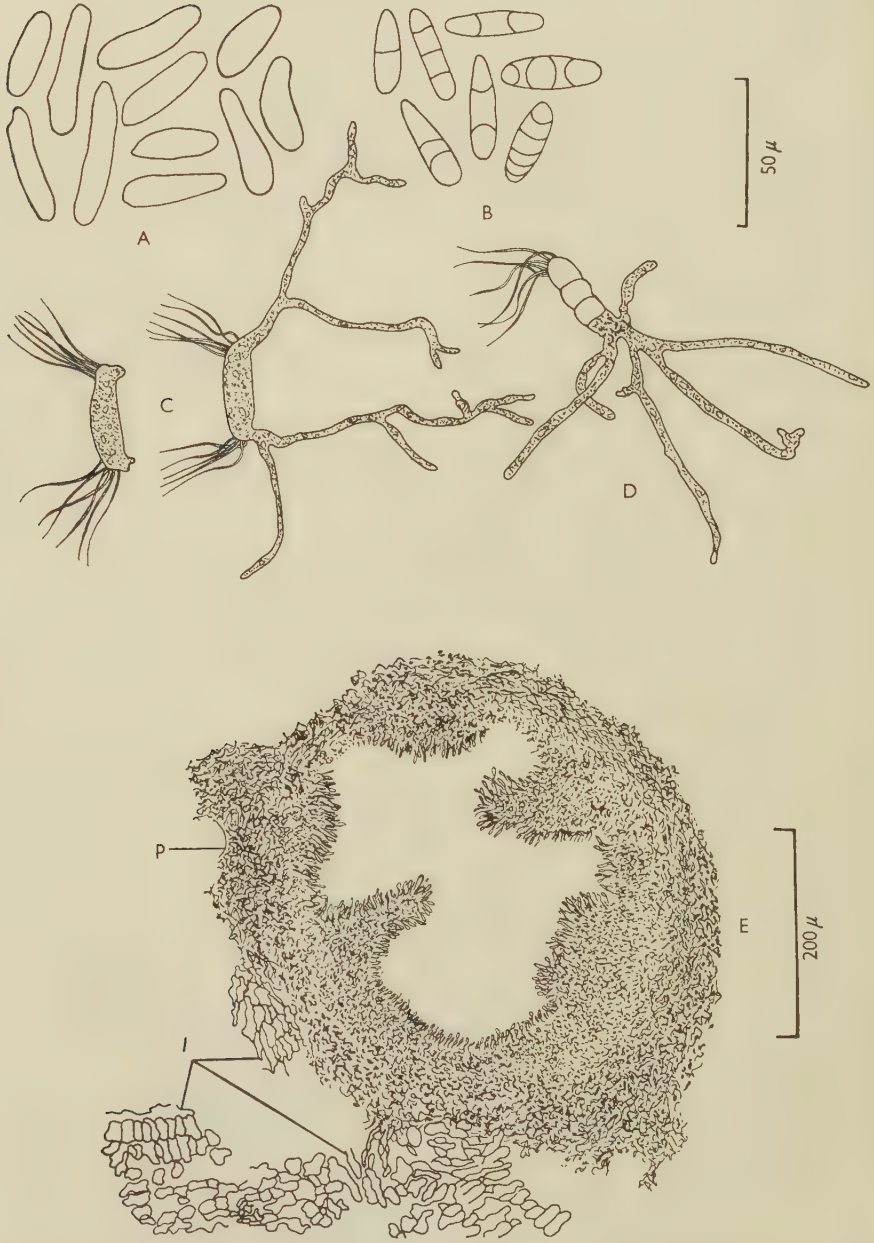
* Since writing this paper I have heard from Miss A. L. van Beverwijk, Centraalbureau voor Schimmelcultures, Baarn, that she isolated this fungus in Switzerland, also from decaying alder leaves in water. Her strain sporulates readily on oatmeal agar.




Text-fig. 1. *Chaetospermum chaetosporum*. A, Three spores from 3-week-old pycnidium; B, septate spore from a 3-month-old pycnidium; C, end view of spore; D, early stages in spore development; E–G, spores at various stages of development showing growth of appendages. A–C, ×550; D–G, ×875.




Text-fig. 2. *Chaetospermum chaetosporum*. A–C, Portions of pycnidial wall from crushed pycnidia of different ages. × 390.




Text-fig. 3. *Chaetospermum chaetosporum.* A, Spores of unusual shape; B, septate spores from 3-month-old pycnidia (appendages are not shown in A and B); C, germinating spores 3 and 12 hr. old; D, germinating septate spore, 18 hr.; E, longitudinal section of pycnidium growing on leaf of alder; *l*, leaf tissue; *p*, pycnidium. A–D, × 390; E, × 142.

yellowish with age. The inner layers of the wall are composed of densely packed hyphae, while the outer layers are rather loosely interwoven, mycelial strands radiating out from the surface of the pycnidium.

The lining branched conidiophores may form tufts projecting into the cavity of the pycnidium (Text-fig. 3E, Pl. 24, fig. 2). The mature spore is hyaline, consisting of an ovoid or elongated, typically unicellular part (28–53 $\mu \times$ 13–15 $\mu$) which is straight or slightly curved, and of polar groups of empty hyaline setae (35–68 $\mu \times c.$ 1 $\mu$). The main body contains granular protoplasm with a few small vacuoles. At either pole 3–11 setae occur, sometimes arranged symmetrically, but more often set slightly to one side of the pole (Text-fig. 1A, Pl. 24, fig. 3). The structure of the spore is exactly as figured by Patouillard; apart from these figures no illustrations of details of structure have been found in the literature.

The conidia are formed from the ends of branched hyphae, each swelling to form the spore primordium. The base of this may grow out on one side or not, so that when, at a slightly later stage, a delimiting wall is formed, attachment of the spore to its conidiophore may be terminal or slightly lateral (Text-fig. 1D). No sooner is the septum laid down than the appendages begin to develop (Text-fig. 1E). At an early stage they are blunt and may occasionally be swollen at the apex, but as they mature the tapered form is established. In the young appendage the protoplasm is dense with numerous vacuoles, but as maturation proceeds the protoplasm seems to be withdrawn and the appendage has the empty appearance characteristic of the mature spore. A cross-wall is never formed at the base of an appendage.

From a study of pycnidia at different stages of development, it seems that the mature spores are carried out into the central region of the pycnidium by the elongation of the conidiophores (Text-fig. 2A–C). When ripe the spores become detached and lie within the cavity of the pycnidium. Spores in pycnidia 3–4 weeks old are usually unicellular and only rarely have a septum or two. However, pycnidia 3–4 months old contain a greater number of septate spores, sometimes with as many as 5 cross-walls (Text-fig. 3B). When the spore is septate, usually only one of the cells, an end cell, contains granular protoplasm.

Germination occurs in water or on nutrient medium, a single germ-tube arising laterally from each end of the spore (Text-fig. 3C).

Patouillard (1888) and most of those who followed him considered the fructification a sporodochium. My own observations of the structures on the leaf and in section show clearly that it is essentially a pycnidium without, however, a differentiated ostiole. It finally ruptures at one side irregularly and may then appear to be a sporodochium.

Patouillard described and figured minute droplets within the basal parts of the setae. I have not observed such droplets within the setae but in crushed preparations droplets, probably oil, liberated from broken hyphae tend to adhere to the outside of the setae near their attachment to the spore (Text-fig. 1G).

Höhnel (1907) described a fungus growing on stems of *Artemisia*, and although he considered the conidia to be similar to those of Patouillard's *T. chaetospora*, he described it as *Pestalozziella ambigua*. Subsequently (1924),

after further examination, he concluded that the fruiting structure was a pycnidium. He further considered that Patouillard's species was also essentially pycnidial and that the two species were co-generic. However, it may well be that only a single species is involved. The spore measurements given by Patouillard were 38–43 × 8–10 $\mu$ and by Höhnel 28–48 × 8–12 $\mu$. A third species *Chaetospermum carneum* with very much smaller conidia (14–16 × 2–4 $\mu$) was described by Tassi (1900). The spores of a pycnidial fungus *Ciliospora gelatinosa* Zimmermann (1902) show some resemblance to those of Patouillard's; Petch (1943) was doubtful if *Ciliospora* is distinct from *Chaetospermum*.

Cultures and specimens have been deposited at the Commonwealth Mycological Institute, Kew (Herb. I.M.I. 76793). Cultures have also been sent to the Centraalbureau voor Schimmelcultures, Baarn.

My thanks are due to Prof. C. T. Ingold for his guidance and encouragement and to the Crown Estate Office for permission to collect material from the Great Park, Windsor.

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## EXPLANATION OF PLATE 24

*Chaetospermum chaetosporum*

Fig. 1. Cluster of pycnidia growing on leaf of alder in pure culture. × 24.
Fig. 2. Vertical section of pycnidium on leaf. × 110.
Fig. 3. Four living spores. × 410.

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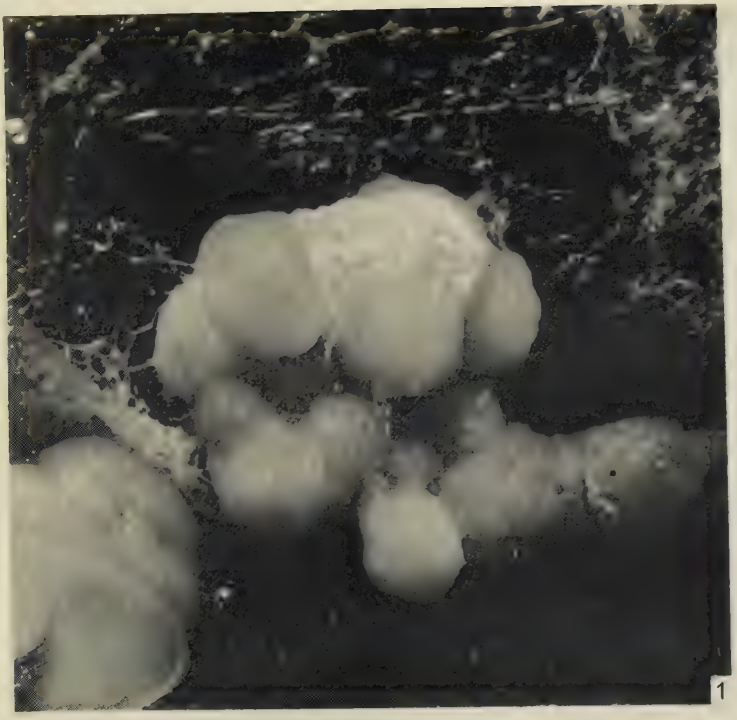


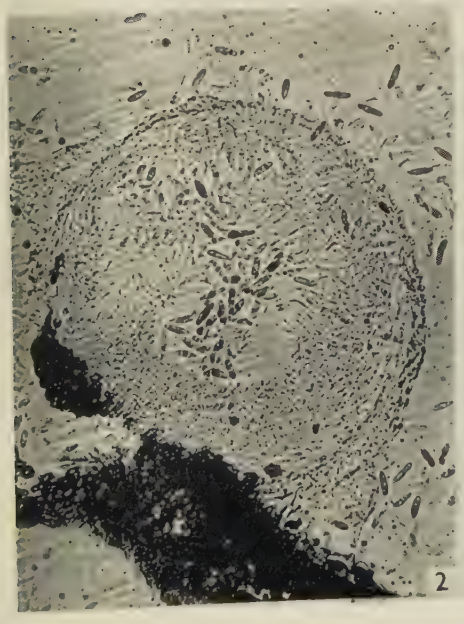

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# THE RE-DISCOVERY OF *GYMNOASCUS GYPSEUM*, THE PERFECT STATE OF *MICROSPORUM GYPSEUM*, AND A NOTE ON *TRICHOPHYTON TERRESTRE*

By D. M. GRIFFIN

*School of Agriculture, University of Sydney, New South Wales*

(With Plates 25 and 26)

The re-discovery of *Gymnoascus gypseum* Nannizzi, the perfect state of *Microsporum gypseum* (Bodin) Guiart & Grigoraki, is described; it was isolated from virgin bushland soil from Turramurra, N.S.W., Australia. An emended diagnosis is provided. The production of 'abortive cleistothecia' by *Trichophyton terrestre* Durie & Frey is described.

There has been controversy concerning the sexual states of the dermatophyte fungi for many years, and reference may be made to the reviews by Tate (1929), Gregory (1935), and Langeron & Vanbreuseghem (1952) for the details. Of immediate importance is the paper by Nannizzi (1927) in which is described the production of cleistothecia by *Microsporum gypseum* (Bodin) Guiart & Grigoraki, when that fungus was grown on old hide fragments and chicken feathers mixed with moist soil. Nannizzi gave the name *Gymnoascus gypseum* to this perfect state of *M. gypseum*, but his work was severely criticized by Langeron & Milochevitch (1930) and others, largely on the grounds that the substrata used were not sterilized and that the cultures were thus impure. This assertion was explicitly refuted by Pollacci (1931), Professor of the Botanical Institute, Royal University of Siena, at which Nannizzi was working, though there is certainly no mention of sterile substrata in Nannizzi's paper. As far as I know, these experiments have never been successfully repeated, and it has been generally assumed that Nannizzi was mistaken, his *G. gypseum* being in fact a chance association of *M. gypseum* with a previously unknown gymnoascomycete.

In April 1959 I by chance came across what I believe to be *G. gypseum* during a study of the successions of fungi on natural substrata decomposing in contact with soil. In this instance human hair sterilized with propylene oxide (Hansen & Snyder, 1947) was placed on the surface of moist, virgin bushland soil from Turramurra, N.S.W., in a Petri dish on a laboratory bench. After 25 days the succession was largely completed and the hair was being rapidly colonized by the final colonizer, *M. gypseum*. Twenty-eight days later small, white spherical bodies were seen in association with *M. gypseum*. These proved to be cleistothecia typical of the Gymnoascaceae. Under the microscope, in more than twenty instances, macroconidia of *M. gypseum* were observed to arise from the distinctive peridial hyphae of the cleistothecium (Pl. 26, fig. 7), proving that only one organism was giving rise to both sexual and asexual states.

In culture on the normal agar media or on sterile hair resting on moist cotton-wool or agar in a Petri dish, only the conidial state was produced, both from cultures derived from macro-conidia and from ascospores. However, when the conditions of the original experiment were simulated, cultures derived from either spore-type produced cleistothecia. Soils from two localities were placed in separate Petri dishes and autoclaved at 15 lb. pressure for 1 hr. After cooling, the soil was moistened with sterile water and sterile hair was placed on the surface. The dishes were then inoculated and incubated at 25° C. After 3 weeks the hair was covered by a weft of mycelium and a few small immature cleistothecia had formed on all plates. After 7 weeks many cleistothecia were present, with the same characteristics as those of the original specimens. It would therefore seem to be necessary for the fungus to be grown on keratin substrata mixed with moist soil if cleistothecia are to be produced.

It is uncertain whether the present isolate is homo- or heterothallic. The few certainly *mono*-ascospore cultures grown have produced typical cleistothecia but without asci—an occurrence also sometimes found in some multi-ascospore or conidial inoculations. The evidence therefore points towards heterothallism but is not yet conclusive.

When the shaved back of a guinea-pig was inoculated by rubbing with the fungus, a lesion developed within 10 days. Hyphae were visible within the scales and the fungus was re-isolated from them.

For reasons given in the discussion below, I have felt it necessary to emend Nannizzi's diagnosis in certain important details and the emended diagnosis is now presented.

### *Gymnoascus gypseum* Nannizzi emend. Griffin

*Cleistothecia* (Pl. 25, fig. 1) spherical, white when immature, buff to light brown when mature (largely coloured by the ascospores and macro-conidia), 300–1000 $\mu$ diam. *Peridium* of interwoven, branched, hyaline hyphae, *ca.* 5 $\mu$ diam.: terminal branches of peridial hyphae curved, 21·5–50 $\mu$ long (excluding appendages), 5–6 $\mu$ diam., with somewhat thickened walls, densely asperulate; individual cells 8–21·5 (mostly *ca.* 14) $\mu$ long, with marked constrictions at the ends and frequently at one or two places along the length, occasionally almost iso-diametric and asymmetrically 'dumb-bell' shaped (Pl. 25, figs. 2, 3, 5). These branches end either in a blunt point or a septate, thin-walled appendage, 1·5–2·0 $\mu$ diam., generally in the form of a spiral of 4–18 (usually 8) turns (Pl. 25, figs. 3–5), the spirals usually lying within the peridium. Cells of inner peridial hyphae similar to those of the terminal branches but with characteristic asperulation and constrictions less marked (Pl. 25, fig. 4). Ascogenous hyphae very thin. *Asci* densely clustered, borne laterally on the ascogenous hyphae, globose, 8 spored, wall evanescent, *ca.* 5·7 $\mu$ diam. (Pl. 25, fig. 6). *Ascospores* pale golden-yellow, flattened-oblate, 2·2–3·2 $\mu$ diam. in polar view, 1·4–2·0 $\mu$ broad in equatorial view, smooth (Pl. 25, fig. 4; Pl. 26, fig. 9). Cleistothecia produced on keratin substrates mixed with moist soil.

Conidial state of (1) many large, 5–7-septate, ellipsoid, rough-walled

aleuriospores (macro-conidia) (Pl. 26, fig. 8), 30–56 × 12–15 μ, either terminal or lateral on both ordinary mycelium and peridial hyphae (Pl. 26, fig. 7), and (2) small, smooth, unicellular, clavate aleuriospores (micro-conidia), 3–9 × 2·4–4 μ, lateral, sessile or nearly so, on mycelium. (Conidial state *Microsporum gypseum*.)

Radial growth in a Petri dish at 25° C. is 2·7–2·8 mm./day on 2 % potato dextrose agar, 2·4 mm./day on Sabouraud's dextrose agar. Mature colony buff to light brown, powdery, tending to become pleomorphic with age. Keratinolytic, causing dermatomycosis in man and many animals.

The new isolate is deposited at the Commonwealth Mycological Institute, Kew (Herb. I.M.I. no. 80558) and a subculture maintained in the Plant Pathology Culture Collection at the School of Agriculture (PPUS 222).

## Discussion

Although I consider the fungus isolated by me to be conspecific with Nannizzi's, there are differences at a number of points which have caused me to emend his diagnosis and which call for some comment.

The difference in colour of the cleistothecium—'white' (Nannizzi), 'white when immature, buff to light brown when mature' (D.M.G.)—does not seem to be very significant because the colour of the mature cleistothecium seems to be largely due to the colour of enclosed ascospores and attached macro-conidia. Again, Nannizzi makes no mention of the spiral appendages to some of the peridial hyphae but I think these may have been overlooked, for in many cleistothecia they are few and never as abundant as in *Shanorella* Benjamin (1956). The difference in shape of ascospores is less easy to reconcile ('oval'—Nannizzi); those of the present isolate are approximately oval in equatorial view, but definitely circular in polar.

Of far greater importance are the numerous discrepancies in dimensions, and the issue is confused by a marked difference between dimensions given in Nannizzi's diagnosis and those which can be calculated from the figures and given magnifications in his paper (Table 1).

Table 1. *Dimensions recorded for various structures in* Gymnoascus gypseum

| | Nannizzi | | |
|---|---|---|---|
| | Diagnosis | Drawing | D.M.G. |
| Outer peridial hyphae max. diam. (μ) | 6–8 | 5·4 | 6 |
| Inner peridial hyphae max. diam. (μ) | 4–5 | 4·3 | 5 |
| Ascus diam. (μ) | 7–8 | 5·6 | 5·7 |
| Ascospore size (μ) | 4·5–3 (*sic*) | 2·2–2·5 × 1·6–1·7 | 2·2–3·2 × 1·4–2·0 (equatorial) view) |

The most serious discrepancies between the dimensions as obtained by Nannizzi and those obtained by myself, i.e. for asci and ascospores, disappear if the values calculated from Nannizzi's drawings are used. It would therefore appear that some error was introduced in the derivation

of the data in his diagnosis and I have substituted my own in the emended diagnosis.

Concerning the correct generic placement of the species, I feel uncertain. As recently reviewed by Benjamin (1956) and Kuehn (1956, 1958) *Gymnoascus* Barenetzky is characterized, amongst other things, by anastomosing peridial hyphae and the absence of spiral appendages. Now the present fungus does not have anastomoses but it does have spiral appendages so that its inclusion in *Gymnoascus* is difficult and must be rejected if these characteristics are considered obligate. Of the other genera in the family, only *Arthroderma* Berkeley is remotely similar and '*gypseum*' could be included within it if the generic limits for this, at present monotypic, genus were drawn sufficiently widely to include those species with peridial hyphae markedly constricted at the septa, and possibly at other places, so that some, at least, of the cells were 'dumb-bell'-shaped. *Arthroderma* is characterized also by spiral appendages and clavate micro-conidia. In many ways the recently described perfect state of the closely allied *Keratinomyces ajelloi* (Dawson & Gentles, 1959) seems intermediate between '*gypseum*' and *A. curreyi*. The only other alternative is to erect yet another monotypic genus in a family already well blessed with them—a circumstance which leads to the speculation as to whether generic limits are not being too finely drawn. I have, therefore, left '*gypseum*' in *Gymnoascus* until the situation becomes clearer.

This rediscovery provides confirmation of the widespread view that the dermatophytes have gymnoascomycete affinities, a view supported by the discovery of the perfect state of the keratinolytic saprophyte *K. ajelloi*. It also supports the views of Grigoraki (1925) and Nannizzi (1926, 1930) that the spiral and antler-like hyphae and other structures frequently found in the saprophytic phase of dermatophytes may be interpreted as the final stage of degeneration of cleistothecia. The scattered spiral and asperulate peridial hyphae are extremely well depicted (as *Trichophyton gypseum*) by Davidson & Gregory (1938).

### A note on *Trichophyton terrestre*

The production by dermatophytes of structures apparently identical with gymnoascomycete cleistothecia, except that they contain micro-conidia instead of ascospores, has been noted (Langeron & Milochevitch, 1930). I have recently observed these in *T. terrestre* (Durie & Frey, 1957) grown on hair under the conditions described above for *G. gypseum*.

These structures (Pl. 26, fig. 10) are characterized by very distinctive peridial hyphae, each cell of which is very short and has three, or occasionally four, large protuberances produced at right angles to each other: if there are only three, the part lacking always lies on the inner side of a curved hypha (Pl. 26, fig. 11). Many of the peridial hyphae end in a fine appendage, which becomes spiral when the whole structure is mature, and projects some distance from the edge of the peridium. At maturity the spiral is in some way very sticky (Davidson & Gregory, 1938) and readily attaches itself, along with the 'abortive cleistothecium' to any object which touches it. In this way the mature structure with its mass of enclosed

micro-conidia is well adapted for dispersal. It is interesting to note that the spiral appendages of *G. gypseum* seem to lie wholly within the peridium rather than projecting outside, as described for *T. terrestre*.

As yet, no asci have been found in these structures. Should they be found, *T. terrestre* would certainly reveal itself as a member of the Gymnoascaceae, but again generic placement would not be easy for the peridial hyphae are not exactly similar to those of any described genus, though *Arthroderma* is probably closest.

I would like to acknowledge the assistance of Dr B. R. V. Forbes and Mr M. F. O'Keefe, School of Public Health and Tropical Medicine, Sydney, for performing the animal inoculations; Miss E. B. Durie and Mrs D. Frey, Royal North Shore Hospital, Sydney, for a culture of *T. terrestre*; and the Department of Illustration, University of Sydney, for the photographs.

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## EXPLANATION OF PLATES 25 AND 26

### Plate 25

*Gymnoascus gypseum*

Fig. 1. Cleistothecium. × 75.
Fig. 2. Terminal branches of peridial hypha, showing constricted, asperulate walls of component cells. × 600.
Fig. 3. Outer peridial hyphae with one spiral appendage. × 300.
Fig. 4. Inner peridial hyphae, spiral appendages and ascospores. × 300.
Fig. 5. Peridial hyphae, slightly out of focus, to show asperulation. × 600.
Fig. 6. Ascogenous hyphae, asci and ascospores. × 600.

### Plate 26

Fig. 7. *G. gypseum*. Young macro-conidium borne on outer peridial hypha. × 600.
Fig. 8. *G. gypseum*. Macro-conidia. × 300.
Fig. 9. *G. gypseum*. Ascospores in polar and equatorial view. × 1500 approx.
Fig. 10. *Trichophyton terrestre*. 'Abortive cleistothecium', with characteristic peridial hyphae and spiral appendages. × 75.
Fig. 11. *T. terrestre*. Peridial hyphae, showing large protuberances and micro-conidia. × 300.

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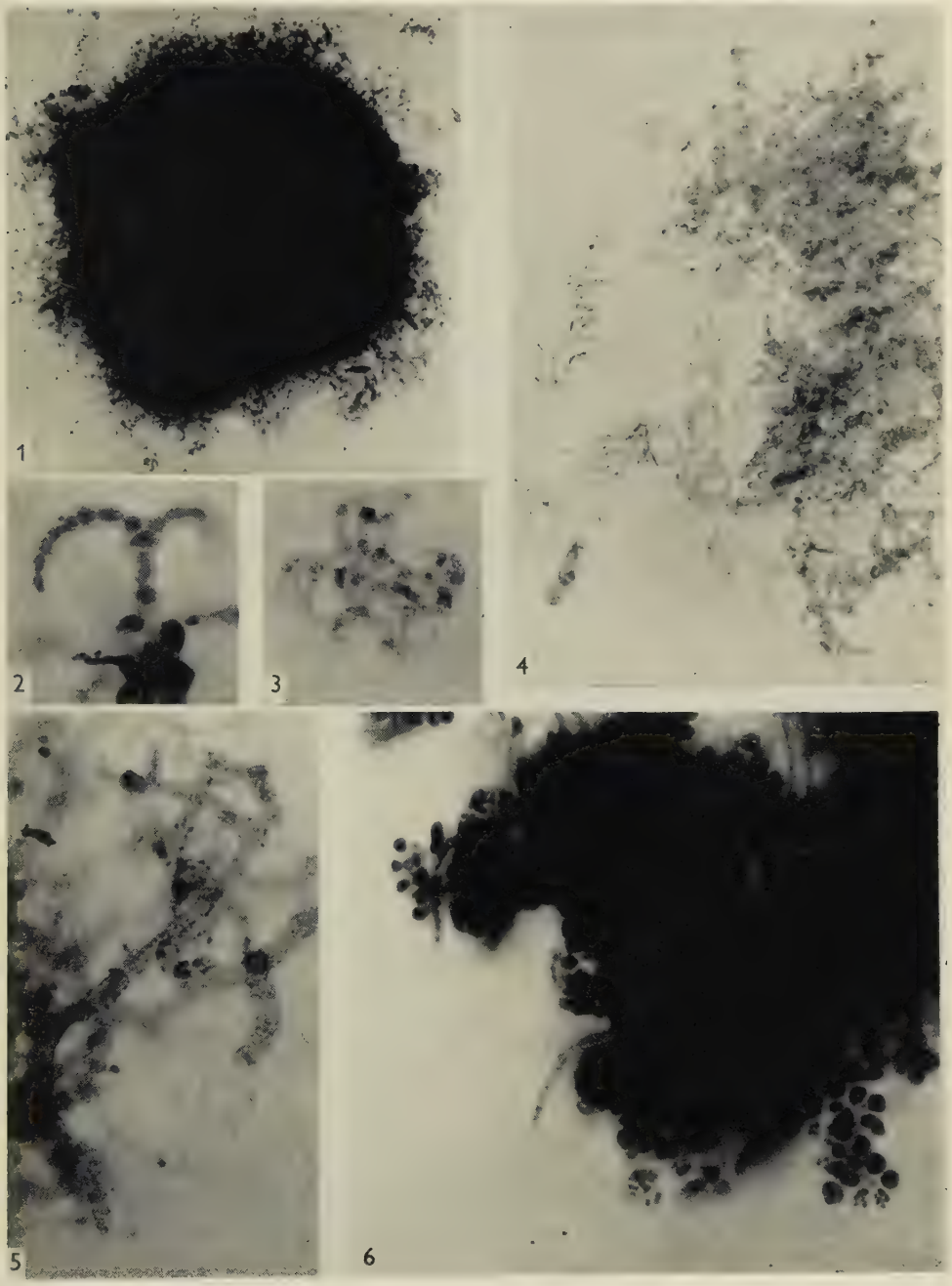


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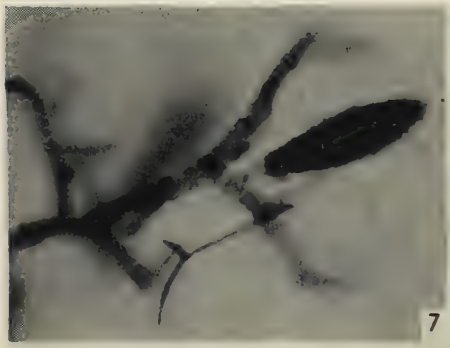
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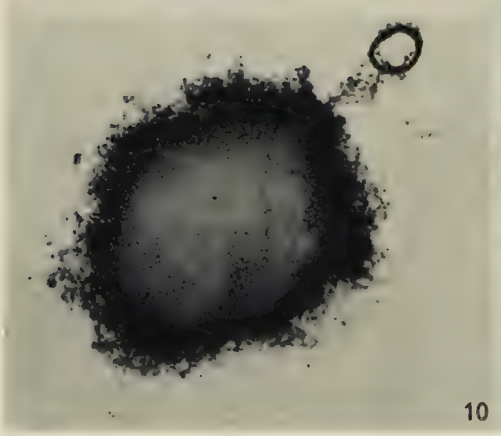
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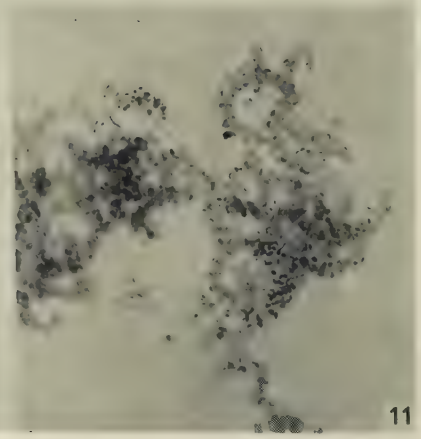
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# INVESTIGATIONS ON A DISEASE OF *COFFEA ARABICA* CAUSED BY A FORM OF *COLLETOTRICHUM COFFEANUM* NOACK.

## II. SOME FACTORS AFFECTING GERMINATION AND INFECTION, AND THEIR RELATION TO DISEASE DISTRIBUTION

By F. J. NUTMAN and F. M. ROBERTS

*Coffee Berry Disease Research Unit, Department of Agriculture, Kenya*

(With 13 Text-figures)

The optimum temperature for germination of the conidia of *Colletotrichum coffeanum* under conditions of low nutrition was around 22° C., but the optimum broadened towards the higher temperatures with improved nutrition. The optimum temperature for lesion formation on green coffee berries was rather lower than that for germination of conidia. The range over which it is possible for germination and infection to occur lies between 17 and 28° C.

Although germination and infection can take place under humidity conditions approaching saturation, the requisite combination of near-saturation with suitable temperatures rarely occurs in the field, and then, usually, for too short a time for infection to be completed. It is concluded that the presence of liquid water, resulting from either rain or heavy mist, is necessary for infection under field conditions.

This study has enabled an explanation to be advanced for the apparent restriction of the disease to certain altitudes, and also for the observed relations between disease incidence and such factors as topography and cultural practices, e.g. the use of overhead shade. Consequently, standard meteorological data can be used to predict the probable incidence of the disease in any given district.

The disease of coffee berries caused by a pathogenic form of *Colletotrichum coffeanum* is now endemic in most of the long-established coffee-growing districts of Kenya Colony above altitudes of about 5500 ft. and is spreading at similar altitudes in more recently planted areas. Outbreaks have been observed below this limit, one at 4900 ft., but these are rare; moreover, they usually occur where local climatic conditions approximate to those of the higher altitudes. Instances are known where, on sloping land, one plantation may experience heavy and consistent attacks while another, a mile or so away, and a few hundred feet lower, either escapes infection or has only light and sporadic attacks.

The fact that very susceptible varieties become infected at altitudes considerably lower than 5500 ft. indicates that this escape is not the result of absence of the pathogen. Also, Nutman & Roberts (1960) showed that one of the most susceptible of the crop-stages is constituted by the developing flowers, and there is evidence that severe loss at this stage may occur, under certain conditions, at the lower altitudes.

The foregoing suggests that certain environmental requirements of

the fungus may be critical, and has led to this study of its nutrient, moisture, and temperature needs associated with germination and infection.

## Techniques

Germination was studied in hanging drops in Van Tieghem cells, on slides coated with agar, and on berry surfaces, using a celloidin stripping technique.

Temperature reactions were studied by means of an apparatus which consisted of nine insulated chambers mounted on the surface of an 18 in. wide strip of steel plate. A thermostat tank was welded to one end, and from the other a metal tongue projected into the freezing chamber of a refrigerator. This gave a range of temperatures from 14 to 35° C. As a rule, only five or six of the chambers, which gave temperatures nearest to those required, were used in each experiment. Heating being from the base, slight vertical temperature gradients occurred in the chambers, and considerable trouble was experienced with distillation to and from hanging drops at the higher and lower temperatures respectively. This was eventually minimized by placing the slides in closed plastic boxes, previously kept in the chambers to equalize the temperatures, and standing them on insulating material to avoid heating or cooling from the base.

Germination on agar surfaces was studied by pipetting shallow pools of the medium on to microscope slides and drawing off the surplus in such a way as to leave an almost plane surface. These films, when 'seeded' with a suspension of conidia, were placed in the plastic boxes lined with moist cellulose wadding, and subjected to the selected temperatures. After the desired time-interval, and in order to minimize lateral movement of ungerminated conidia, the conidia were killed by placing a number of very small droplets of cotton blue in lacto-phenol on the agar surface. When sealed with glass cover-slips and ringed with molten petroleum jelly, the slides could be preserved in a state fit for conidial counts for several days. It was noticed that, although the distribution of conidia on the slides must have been random, there was a very marked tendency for germination to occur in groups; because of this it was found necessary to count very large numbers to ensure accuracy.

In all except the very early experiments the criterion of 'positive' germination, on agar or on berry surfaces, was the presence of an appressorium, or of the club-shaped swelling indicating that one was in the process of formation.

Owing to the very great variation in viability of different batches of conidia (Nutman & Roberts, 1960) data from different tests cannot be pooled. A series of temperature/germination experiments was selected, therefore, covering a wide range of viability, and divided into two groups, one giving a germination rate lower than 20 %, and one higher. All rates were then expressed as a percentage of the maximum for the particular batch, and the data from each group were bulked. Analysis showed that the two groups were, in fact, a single population. It is thus possible to combine information from all comparable trials, resulting in a large increase

in accuracy. For example, Fig. 2 is a result of the examination of over 280,000 conidia: such numbers would not, of course, be practicable in a single experiment.

## Experimental work

### *Effect of temperature on germination of conidia in water*

Fig. 1 combines the results of forty-eight experiments comprising counts of 101,000 conidia. There is a well-defined optimum for germination at around 22° C., with a sharp fall-off at higher and lower temperatures. Above 25° C. germination becomes increasingly abnormal, with long and contorted germ-tubes. Even though each point on the curve is derived

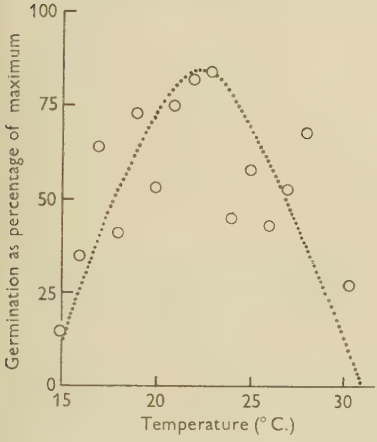


Fig. 1

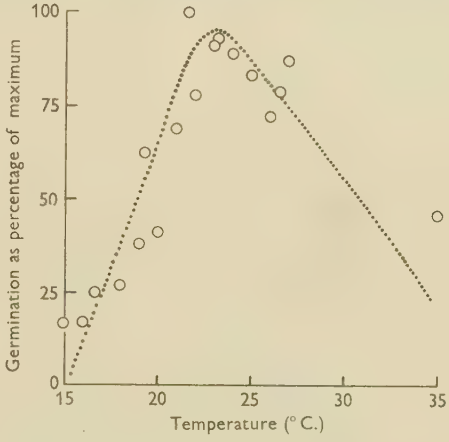


Fig. 2

Fig. 1. Germination of conidia of *C. coffeanum* in water. Forty-eight experiments: 101,000 conidia counts.

Fig. 2. Germination of conidia on potato dextrose agar. Thirty-five experiments: 286,700 conidia counts.

from 6800 counts, the irregularity is marked. Fig. 7 shows a curve derived from six further experiments involving counts of 24,000 conidia. The optimum here lies at about 21° C., and the reduction in germination at the warmer end of the gradient is somewhat greater. The main features of the two curves are, however, identical.

### *Effect of temperature on germination on nutrient agar*

Fig. 2 combines the data from thirty-five experiments, involving counts of 286,700 conidia germinated on 'Difco' potato dextrose agar. The region of high germination lies between 22 and 26° C., 50% of maximum germination occurring at about 30° C.—a temperature which is inhibitory in pure water. Fig. 3 combines data from two experiments comprising 144,000 counts. In these, thin films of non-nutrient agar were seeded with conidia suspended in dilute extracts of coffee-berry skins. The curve is very similar to that of Fig. 2, the increase in germination above 25° C. being well marked.

*Effect of temperature on germination on berry surfaces*

Fig. 4 (nine experiments: 352,000 conidia) illustrates germination on berry surfaces. It will be observed that the curve is similar to that on nutrient agar, with a broad optimum between 22 and 27° C., some degrees above that for germination in water. Fig. 10 also illustrates a similar result.

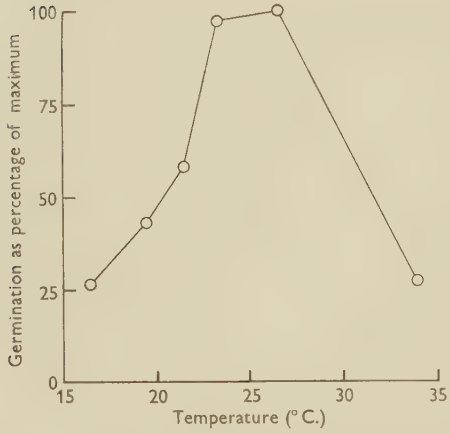


Fig. 3

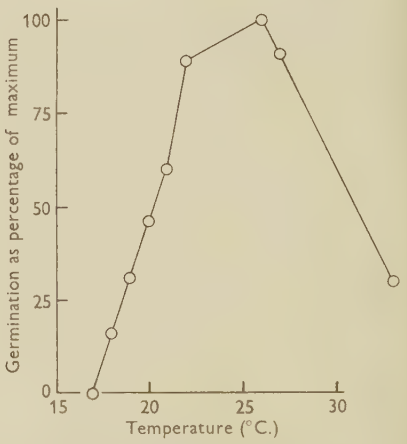


Fig. 4

Fig. 3. Germination of conidia on agar with coffee-skin extracts. Two experiments: 144,000 conidia counts.

Fig. 4. Germination of conidia on berry surfaces. Nine experiments: 352,000 conidia counts.

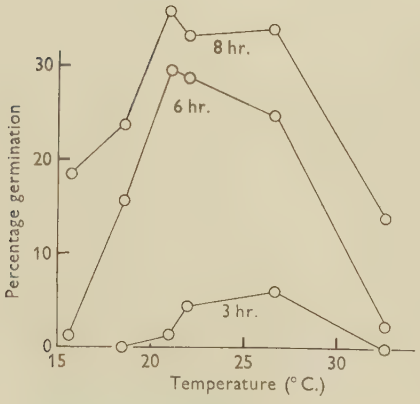


Fig. 5

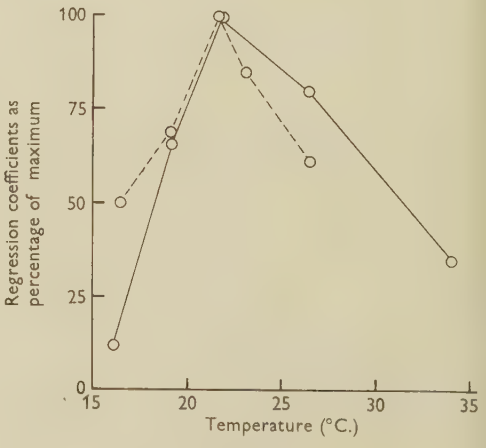


Fig. 6

Fig. 5. Relation between temperature and time of germination of conidia. 68,000 counts.

Fig. 6. Germination of conidia and temperature. Regression coefficients in relation to temperature. Dotted line = series 1, 93,000 counts. Entire line = series 2, 160,000 counts.

*Interaction between temperature and time of germination on nutrient agar*

This was studied by withdrawing a number of slides from the different temperature chambers after varying intervals. During the whole of this series 4000 conidial counts were made for each point. Fig. 5 illustrates the result of one experiment in which the time intervals were 3, 6, and 8 hr., a total of 68,000 counts. The curve, like all high-nutrition ones, has a broad optimum with appreciable germination above 30° C. This germination, however, was abnormal.

All data from this series were treated by calculating, for each experiment, the regression equation for each temperature. The regressions, for time

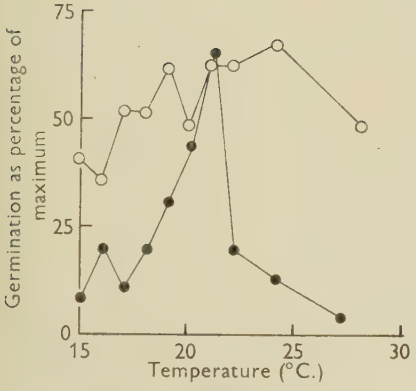


Fig. 7

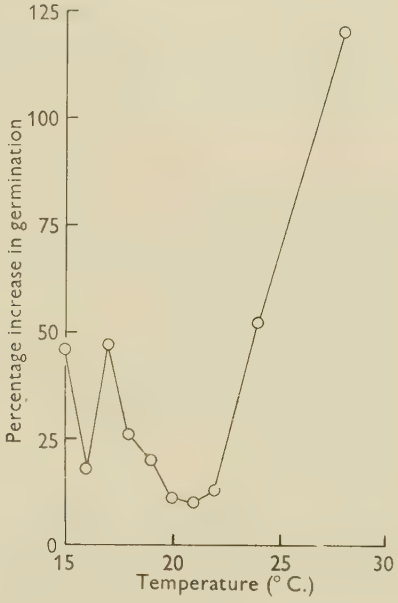


Fig. 8

Fig. 7. Relation between germination of conidia, temperature and nutrition. Black circles = water. Open circles = 1% dextrose. Six experiments: 48,000 counts.

Fig. 8. Relation between temperature and increase in conidial germination rate with 1% dextrose. 48,000 counts.

intervals up to 8 hr., approximate to straight lines. Fig. 6 illustrates, for two large-scale experiments (one comprising 93,000 counts, and the second 160,000 counts), the relationship between temperature and the regression coefficient expressed as a percentage of the maximum for each batch. It is clear that the maximum accelerating effect of temperature on germination in both experiments must be very close to 22° C. The regression equations can also be used, of course, to calculate the germination percentage after any period up to about 8 hr. When this is done, and the curve superimposed on, for example, that in Fig. 2 the degree of correspondence is very high.

*Interaction between temperature, nutrition and germination*

The interaction between temperature, nutrition and conidial germination was studied by determining the rates at two nutritional levels, 0 and 1 % dextrose, and at a range of temperatures. A wider range of nutritional levels would have been desirable, but was impracticable. Six experiments, involving a count of 48,000 conidia, were completed.

Fig. 7 illustrates the results, which are completely in accord with the foregoing. The curve for plain water agrees closely with that in Fig. 1, but the effect of nutrition is again to broaden the optimum. The increase in germination above 25° C. is particularly well marked. Fig. 8 is derived from the same data treated in a different way: the increase due to the increased nutritional level is calculated for each temperature. This is clearly minimal at the optimum temperature (10 % at 21° C.), and increases greatly at higher and lower temperatures (about 50 % at 15° C., and 120 % at 28° C.).

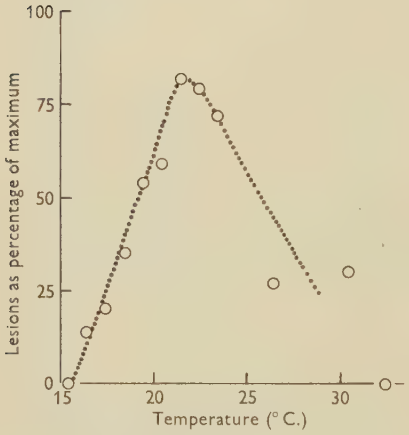


Fig. 9

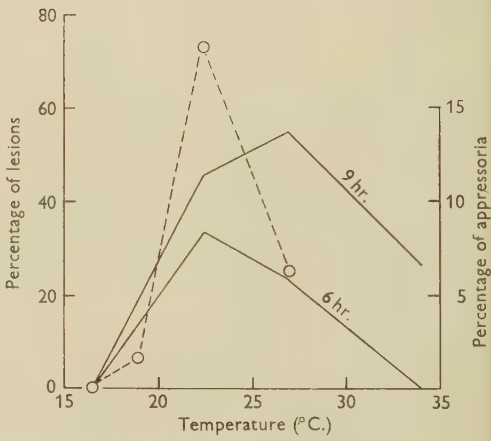


Fig. 10

Fig. 9. Lesion formation in relation to temperature. Eleven experiments: 6300 berries.

Fig. 10. Relation of temperature to formation of appressoria on berry surfaces, and to lesion production on the same sample of berries by the same sample of conidia. Broken line = lesions. Entire line = appressoria.

*Effect of temperature on lesion formation*

Fig. 9 summarizes the results of eleven experiments, involving the inoculation of 6300 berries. A sharp optimum at 21–22° C. is characteristic of each experiment. This is noteworthy in comparison with the data on germination rates on berry surfaces, where the optimum is consistently several degrees higher. A number of experiments were carried out in which lesion-formation and germination at different temperatures were measured on the same batch of berries using the same batch of spores. Fig. 10 illustrates a typical result. The optimum temperature for *germi-*

*nation*, as before, was high; the optimum for *lesion formation* was, as in the main series, at 21–22° C. The importance of this observation on the distribution of the disease will be discussed later.

### *Effect of temperature on mycelial growth*

This was measured in the usual way, on cultures of the fungus on potato-dextrose agar in Petri dishes. Fig. 11 shows that the optimum for mycelial growth lies in the same range as that for conidial germination at a low nutritional level.

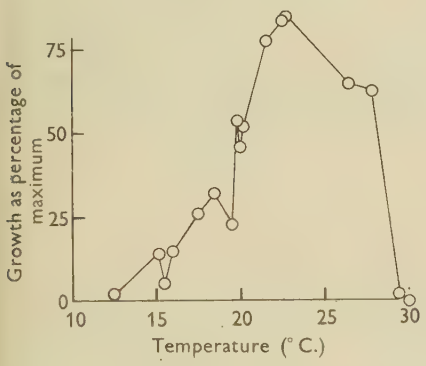


Fig. 11

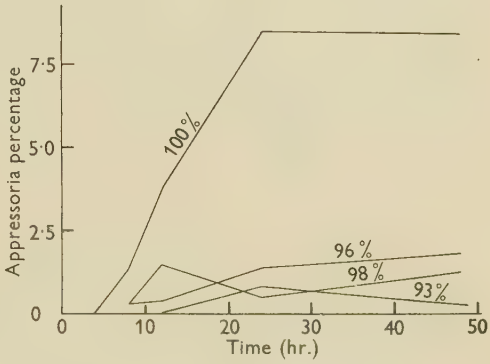


Fig. 12

Fig. 11. Mycelial growth on potato-dextrose agar in relation to temperature.
Fig. 12. Formation of appressoria on berry surfaces in relation to humidity.

### *Effect of nutrition on viability of conidia*

Nutman & Roberts (1960) commented on the great variation in viability of different batches of conidia. The six experiments summarized in Fig. 7 permit of the selection of four covering a wide range of viabilities, this being assessed by maximum germination rate in water. Table 1 shows clearly that the effect of improved nutrition increases as viability decreases.

Table 1. *Effect of intrinsic viability of four batches of conidia on their reaction to a nutritional stimulus*

| | | | | |
|---|---|---|---|---|
| Maximum percentage germination in water | 57·5 | 35·0 | 15·5 | 5·5 |
| Percentage increase with 1 % dextrose | 20 | 70 | 130 | 610 |

The data permit of thirty-five pairs of comparisons (irrespective of time and temperature) between germination rates in water and in dextrose solution. The correlation coefficient is negative and very highly significant ($r = -0{\cdot}458$: $t = 2{\cdot}97$: $P = < 0{\cdot}01$). That this is solely due to a differential reaction to the nutritional stimulus is shown by the fact that correlations within each batch are not significant.

*Effect of humidity on germination*

Different dilutions of sulphuric acid, giving relative humidities of 100, 98, 96 and 93 %, were placed in the standard plastic boxes. Rows of clips, each adapted to hold one berry, were attached to the lids in such a way that the berries were suspended about 5 mm. above the solutions. The berries were previously inoculated with droplets of a conidial suspension which were rapidly dried under a fan before the berries were placed in the humidity chambers. Five berries were withdrawn from each box after 4, 8, 24, and 48 hr. and germination on their surfaces was estimated. The results, illustrated in Fig. 12, show that germination was negligible at any relative humidity below 100 %. It was not possible to observe lesion production in humidities below 100 % since, in the time before these would have become visible, the berries noticeably lost turgidity. Numerous experiments, however, have shown that infection can take place in the absence of liquid water in a saturated atmosphere.

*Effect of desiccation on conidia*

Desiccation in the field will obviously affect: (1) The extruded mass of conidia on the surface of a lesion; in this stage the conidia, bound together in a mucilaginous matrix, may undergo drying conditions for many days before they are dispersed by rain. (2) Single conidia, or small groups, after they have been dispersed by rain.

The effects on undispersed conidia were studied as follows: ripe berries with well-developed lesions were collected in the field and, after washing, placed in damp chambers for 2 days to ensure a copious production of conidia. They were then placed on dry wadding and exposed in the open to full sun, intermittently tempered by tree or cloud shade, for 8 hr. daily. For the remaining 16 hr. of the day the berries were kept in the laboratory. These drying conditions were more severe than those normally encountered in a plantation. Before the berries were first exposed, and on successive days afterwards, five were withdrawn at random and batches of green berries were inoculated with conidia taken from them. Lesion formation was recorded for each batch.

After 1 day's exposure to desiccation, the infectivity of the conidia was consistently and markedly increased by up to 50 %. Subsequently a progressive reduction was evident until, after 10–12 days, the conidia had lost all infectivity. It should be noted that, after 3 or more days of exposure, the standard infection drops produced a number of abnormal lesions, invasions occurring at one or two points only and with the subsequent development of the lesion retarded. In fact, many of these remained of pin-point size only, even after prolonged incubation under optimum conditions. Under field conditions, therefore, it seems probable that 3–4 days of rainless weather, after conidia have been extruded, will reduce their infectivity to a very low level, although a few will remain viable for a much longer period.

The effect of drying on conidia after dispersal, i.e. when they have been

isolated by rain and have lost their mucilaginous coating, was studied by inoculating green berries in the usual way, drying the infection-drops, and keeping them dry for varying periods. The berries were then re-wetted by atomizing water on to their surfaces, and germination and infection allowed to proceed normally. It should be noted that these experiments, made under laboratory conditions, represent a very much milder degree of desiccation than dispersed conidia would encounter in the natural conditions of the plantation.

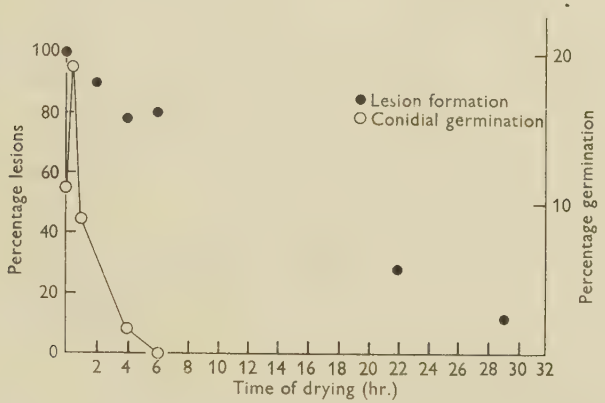


Fig. 13. Germination of, and lesion production by, dispersed conidia after varying periods of mild drying.

Fig. 13 illustrates a typical result. The stimulus to germination by a short period (up to 1 hr.) of drying is a constant feature of all experiments and is in marked contrast to the longer period necessary for stimulation when the conidia are still undispersed in the lesions. Germination falls rapidly to a very low (and unmeasurable) level after 4–5 hr. Infectivity as assessed by lesion production, however, does not cease entirely until after about 30 hr. under conditions of mild desiccation in the laboratory.

A point of some practical importance is whether intermittent periods of suitable conditions for germination can be effective. This was studied by comparing germination and lesion production after varying intervals, and the same but with the interval interrupted by one or more periods of desiccation. The following experiment is typical: five batches of berries were inoculated and treated as follows:

*Batches* 1–3. Incubated in saturated atmosphere for 4, 8 and 12 hr. respectively; infection droplets dried; berries replaced in saturated atmosphere.

*Batch* 4. Incubated 4 hr.; dried for 20 hr.; re-wetted and incubated 4 hr.; dried and re-incubated.

*Batch* 5. As for batch 4, but with addition of another 4 hr. incubation and 20 hr. drying.

At the end of each germination period, ten berries were withdrawn from each batch, and the percentage of appressoria determined (Table 2).

These results, although clear-cut, are not easy to interpret. Conidia

can germinate in a saturated atmosphere, though usually at a lower rate than in liquid water, and it is clear that this has happened in the 'un-interrupted' series to give the high lesion formation rates of 63 and 66 % for the 4 hr. and 8 hr. periods, respectively. The progressive reduction in the 'interrupted' series is as would have been expected. Reference to Fig. 13 shows that a 20 hr. period of drying would reduce lesion formation by between 50 and 60 %, and this is more or less what has happened.

The comparatively high rate of appressorium formation in the 'interrupted' series is difficult to reconcile with the reduced rate of lesion formation. Visual observation, however, showed that a proportion of the conidia in the once-interrupted batch, and a higher proportion in the twice-interrupted, germinated in a somewhat abnormal manner, exhibited a different staining reaction, and appeared empty of contents. It may well be that the periods of drying, although they permitted some subsequent germination, so reduced vigour that penetration and infection were affected.

Table 2. *Effects of interrupted and un-interrupted periods of germination on appressorium formation and lesion production*

| Treatment | Percentage appressoria | Percentage lesions |
|---|---|---|
| 4 hr. germination | 0·5 | 63 |
| 8 hr. germination | 12·4 | 66 |
| 12 hr. germination | 19·4 | 81 |
| 8 hr. germination (once interrupted) | 17·9 | 35 |
| 12 hr. germination (twice interrupted) | 15·0 | 15 |

It seems, therefore, that under the mild conditions of laboratory desiccation, germination can be resumed if suitable conditions recur after a minimum period of 20 hr. drying, but infection is greatly reduced. In the field, however, germination would normally be interrupted by severe insolation, and the effect on infectivity is likely to be correspondingly greater.

## Discussion

This study was undertaken to obtain the basic data which might lead to an understanding of the reasons for the present distribution of the coffee-berry disease, and its relation with altitude and topography; also to reconcile conflicting field observations and, in particular, to attempt to forecast the zones into which the disease might be expected to spread. This is of vital interest in any question of the expansion of the coffee-growing industry.

At present the disease is endemic in most districts of altitudes of 5500 ft. and over, where annual attacks of greater or less severity may be expected. Below this altitude, sometimes with only 400 or less feet involved, is a border-line zone where the disease occurs occasionally, or where only the very susceptible stages of the crop, such as flowers or recently set fruit, are sometimes attacked. A large area under coffee cultivation in the yet lower altitudes escapes the disease. The pathogen is present there at a very low level; highly susceptible varieties are occasionally attacked but

colonization of the maturing cortex of the twigs is almost entirely by the non-pathogenic form of the fungus (Nutman & Roberts, 1960).

A pre-requisite for attack is not only the maintenance of suitable conditions for the right length of time for conidial production, but also that suitable temperature and moisture conditions should persist for long enough afterwards for germination and host penetration to be completed.

Factors involved in the establishment of an epidemic are:

(1) *Presence of a source from which inoculum can be produced.* As will be shown in a later paper, the main source of inoculum is the infected cortex of the twigs, especially in the earlier stages of an outbreak. In the higher altitudes, colonization of these twigs is exclusively by the pathogen, but the proportion of this form to the non-pathogenic form of the fungus decreases with decrease in altitude until the latter predominates. As regards the time necessary for conidial production, experiments have shown that twigs collected in dry conditions from the field require at least 12 hr. in a humid atmosphere before conidia are produced in any significant quantity.

(2) *A mechanism for the transport of conidia, in adequate numbers, to the berry surface*: Bock (1956), and Nutman & Roberts (1960), have both shown that a high concentration of conidia is necessary, infection being greatly reduced below about $10^5$ conidia/ml. It seems obvious that transport over the very short distances involved must be, in the main, by rain-splash. Bock's observation that infection did not occur when berry-bearing branches were protected by polythene bags, inside which inoculum was present, supports this view. It should be noted that invasion of berries by mycelial growth from the stem can cause loss of crop without the intervention of conidia: this, however, is of local importance only, and not a general phenomenon.

(3) *Maintenance of a suitable temperature for a sufficient period.* It has been shown that the optimum temperature for germination and lesion production extends over a fairly wide range, but that attack is greatly reduced at 17° C. and inhibited at 15° C. Above 28° C. germination is, in general, abnormal, and infection greatly reduced. As regards the period required, the absolute minimum is about 3 hr., yet only 9 % of maximum invasion occurs after 5 hr., and even after 10 hr. it is only three times greater. These figures are for optimum temperatures, and the period would be longer at any others. For the purposes of this discussion, therefore, we have taken the period for invasion to be 5 hr. anywhere within the range 17–28° C., and it would seem probable that this approaches a minimum figure.

(4) *Maintenance of suitable moisture conditions for an adequate period.* Our experiments have shown that germination and invasion are negligible at any relative humidity below 100%. The unimportance of relative humidity as a factor in disease incidence in the field is shown from an analysis of temperature and humidity records taken inside a coffee bush on a borderline plantation, where the disease is known to occur mainly on susceptible varieties or under shade. Paired records of temperature and humidity for all but 14 days of the year 1958 were studied. On only one occasion did a suitable relative humidity coincide with a suitable temperature, and

then for a period too short to permit of infection. Kirkpatrick (1935) too carried out very precise observations on another border-line plantation, using an Assmann psychrometer. Study of his data shows that at no time did the relative humidity rise to a sufficiently high level when the temperature was within the limits permitting germination: on seven occasions suitable humidities prevailed, but none of these coincided with suitable temperatures. In addition, studies of thermohygrograph records from other sites, both at high and at low altitudes, confirm the fact that rarely, if ever, does a suitable temperature coincide with a suitable relative humidity. In effect, this means that infection, even in the higher altitudes, can take place only when liquid water is present on the fruit, and relative humidity per se need not be considered further as a factor. Thus attention is directed towards the incidence of rain or heavy mists.

### *Effect of altitude on disease incidence*

Kirkpatrick (1935) made an exhaustive and valuable study of the climate and eco-climates of coffee plantations. His work was carried out on a border-line plantation, and included observations on the relationships between meteorological factors as measured in a Stevenson screen in the open and those inside coffee bushes under various conditions of shade and exposure. In addition to this site we have standard thermograph records for several other districts, both in the severely attacked high altitudes and in the border-line zones. When suitably adjusted, these show that in all the districts studied, night temperatures are too low for germination or infection to take place; only day temperatures, therefore, need be considered further.

In the lower altitudes, maximum temperatures are often very much too high for infection. The rise in the early morning, and the drop in the evening are both very rapid, and, for considerable parts of the year, temperatures remain between the upper and lower limits for invasion, as a general rule, for only 2–4 hr. at a time: this is too short a period for germination and invasion to be completed and, with temperatures frequently in excess of 30° C. and relative humidities averaging less than 50 % (and often as low as 20 %), the desiccation of any un-germinated, or partly germinated, conidia present on the berries during the midday hours would effectively destroy their viability. In these zones, as can be seen in Table 3, unfavourable time/temperature conditions prevail on about half the days. Conditions suitable for the invasion of flowers (where germination and penetration can be completed in little more than 2 hr.), however, occurs with much greater frequency, and loss in this stage of the crop is, in fact, experienced in this zone. The reason for its comparative infrequency is thought largely to be the ephemeral nature of the flowers.

In the higher and severely affected altitudes, however, the situation is very different. Only rarely do maximum temperatures rise high enough to inhibit germination and invasion, and for many hours daily, over prolonged periods, temperatures near the optimum prevail, sometimes remaining between the 17 and 28° C. limit for as long as 16 hr.; moreover, the midday period of maximum temperatures is often too short completely

to destroy the viability, by desiccation, of dispersed conidia. In exceptionally cold spells, which, however, are usually of short duration, temperatures may not rise at all above the minimum for infection. In fact, in these altitudes invasion is possible on 80–90 % of all days so far as temperature is concerned, as compared with 54 % in the border-line areas. This difference, although considerable, seems insufficient in itself entirely to account for the great difference in disease incidence between the high-altitude and the border-line zones; it is clear that other factors must operate, and it is suggested that the most important of these is the amount, and especially the distribution, of the rainfall.

From a study of sixteen estates selected at random, eight subject to severe annual attack and eight disease-free, the following facts are relevant:

*Estates subject to disease.* Average altitude, 6474 ft.; average rainfall (21–58 yr. records), 52·5 in.

*Estates with no disease.* Average altitude 5156 ft.; average rainfall (12–47 yr. records), 36·2 in.

Table 3 presents temperature and rainfall data for three dissimilar districts. It shows the number and percentage of days for each month on which temperatures suitable for infection prevail for 5 hr. and longer: the number and percentage of these on which rain fell: and the number and percentage on which rain fell also on the preceding day. Unfortunately we do not know how often the periods of rainfall coincided with the periods of suitable temperatures. The probability is that they frequently did do so, but the figures in the table may well be too high. The first district is on the west side of the Rift Valley, and heavy and consistent attacks have been experienced there since the first appearance of the disease. The second, east of the Rift, is also seriously, but less consistently, attacked. The third escapes serious attack, although the disease is present at a very low level.

It can be seen in Table 3 that in District I (6500 ft.), suitable combinations of temperature and rainfall occur frequently during all periods of the year for which information is available: in December conditions seem to be more adverse, but we have records for only 13 days of that month. District II, 300 ft. lower, is similar, and differs only in the lengthy period (June–September) during which infection cannot take place; the number of days in the year, however, on which conditions are suitable is about the same. District III (5400 ft.) has less than half the number of potentially infective days, and for many months infection is extremely improbable, either because of unsuitable temperature, or unsuitable moisture conditions, or both.

Although these data seem adequate in themselves to account for the gross difference in disease incidence between the two first districts, on the one hand, and the third district, on the other, an additional factor is known to operate. Mycelial growth and lesion development have approximately the same temperature-requirements as have conidial germination and infection of the host. In the first two districts, the development of the pathogen within the tissue of the plant can proceed unchecked, in conditions near the optimum, for many more hours of each day than in the third district. Consequently any seasonal build-up of the pathogen would

Table 3. *Comparison between the climatological régimes of three districts varying with respect to incidence of disease*

*District I*

West Rift: heavy and consistent disease incidence; alt. 6500 ft.

| | (1) | (2) | % | (3) | % | (4) | % |
|---|---|---|---|---|---|---|---|
| Jan. | — | — | — | — | — | — | — |
| Feb. | — | — | — | — | — | — | — |
| Mar. | — | — | — | — | — | — | — |
| Apr. | 29 | 17 | 59 | 8 | 28 | 4 | 14 |
| May | 30 | 18 | 60 | 7 | 23 | 4 | 13 |
| June | 23 | 9 | 39 | 5 | 22 | 2 | 9 |
| July | 30 | 27 | 90 | 11 | 37 | 5 | 17 |
| Aug. | 29 | 22 | 76 | 14 | 48 | 8 | 28 |
| Sept. | 25 | 25 | 100 | 12 | 48 | 7 | 28 |
| Oct. | 28 | 25 | 89 | 6 | 21 | 3 | 11 |
| Nov. | 16 | 16 | 100 | 2 | 13 | 1 | 6 |
| Dec. | 13 | 13 | 100 | 1 | 8 | 0 | 0 |
| Mean | — | — | **79** | — | **28** | — | **14** |

*District II*

(East Rift: heavy and consistent disease incidence; alt. 6200 ft.)

| | (1) | (2) | % | (3) | % | (4) | % |
|---|---|---|---|---|---|---|---|
| Jan. | — | — | — | — | — | — | — |
| Feb. | 12 | 12 | 100 | 1 | 8 | 0 | 0 |
| Mar. | 31 | 31 | 100 | 9 | 29 | 6 | 19 |
| Apr. | 30 | 30 | 100 | 12 | 40 | 7 | 24 |
| May | 31 | 31 | 100 | 20 | 65 | 17 | 55 |
| June | 30 | 29 | 97 | 1 | 3 | 0 | 0 |
| July | 31 | 17 | 55 | 0 | 0 | 0 | 0 |
| Aug. | 31 | 22 | 71 | 0 | 0 | 0 | 0 |
| Sept. | 30 | 27 | 90 | 2 | 7 | 0 | 0 |
| Oct. | 31 | 31 | 100 | 2 | 7 | 1 | 3 |
| Nov. | 30 | 30 | 100 | 16 | 53 | 12 | 40 |
| Dec. | 27 | 25 | 93 | 4 | 15 | 3 | 11 |
| Mean | — | — | **91** | — | **21** | — | **14** |

*District III*

(East Rift: disease of local and rare occurrence; alt. 5400 ft.)

| | (1) | (2) | % | (3) | % | (4) | % |
|---|---|---|---|---|---|---|---|
| Jan. | 25 | 11 | 44 | 1 | 4 | 0 | 0 |
| Feb. | 28 | 10 | 36 | 1 | 4 | 0 | 0 |
| Mar. | 31 | 13 | 42 | 6 | 19 | 5 | 16 |
| Apr. | 30 | 17 | 57 | 4 | 13 | 3 | 10 |
| May | 31 | 21 | 68 | 9 | 29 | 7 | 22 |
| June | 30 | 20 | 67 | 4 | 13 | 1 | 3 |
| July | 27 | 9 | 33 | 0 | 0 | 0 | 0 |
| Aug. | 31 | 25 | 81 | 0 | 0 | 0 | 0 |
| Sept. | 30 | 11 | 37 | 0 | 0 | 0 | 0 |
| Oct. | 31 | 9 | 29 | 0 | 0 | 0 | 0 |
| Nov. | 30 | 20 | 67 | 10 | 33 | 5 | 16 |
| Dec. | 31 | 27 | 87 | 7 | 22 | 3 | 10 |
| Mean | — | — | **54** | — | **11** | — | **6** |

Column (1) Number of days for which records are available.
(2) Number of days with temperatures suitable for invasion by *C. coffeanum* for not less than 5 continuous hours.
(3) As above, and with rain on the same day.
(4) As above, but with rain also on the preceding day.

be much slower in the lower region, and, in fact, its level is demonstrably lower.

Included in the group of eight severely attacked plantations, to which reference has been made above, is an apparently aberrant one. This, at an altitude of 6200 ft., has a rainfall of only 32·6 in. No thermograph data are available for it, but its temperature régime would probably approximate to that of the second district cited in Table 3. Its rainfall, however, is even lower than that of the third district (38·2 in.), and consequently, on this factor alone, it would be expected to escape attack. The plantation is, however, situated on the high ground between Mt. Kenya (17,058 ft.) and the Aberdare Mountains (about 13,000 ft.) and is subject to prolonged periods of heavy mist which would be amply sufficient to enable conidial production to take place on most days. This, it is thought, adequately accounts for its disease incidence. Rayner (1952), too, mentioned the 'high humidities associated with misty conditions' as the probable cause of disease in this area.

*Effect of topography on disease incidence*

It has been generally observed that the disease is worse in hollows, low-lying areas, valleys, and on western aspects (Rayner, 1952). So far as the hollows are concerned, Rayner cites Macdonald (1926) as having associated the greater incidence of disease in such situations with higher humidities, while he himself thought that it was the lower night temperatures which were responsible. The low temperatures induced by hail were also associated by Rayner with the often-observed subsequent increase in disease. We consider, however, that the twofold effect of wounding by hail impact —namely, the reduction in intrinsic berry resistance, and the stimulus to germination resulting from nutrients being liberated from the berry through the wound, is amply sufficient to account for the observed disease increase (Nutman & Roberts, 1960).

Kirkpatrick (1935) showed that the effect of hollows is to lower the minimum temperatures and to raise the maximum. The experiments reported here have shown that night temperatures on level ground are rarely, if ever, high enough for spore production or germination to take place, and any decrease due to hollows, therefore, could have no effect.

Thermograph records obtained for a region where the disease is unusually severe (District I of Table 3) show that on only 31 % days of the year did the maximum temperature exceed the optimum for invasion, and then never by more than 2° C. Even in unshaded coffee, therefore, and especially on rainy days, the daytime temperatures must often be well below the optimum, although above the minimum for invasion. Temperatures in bushes in a hollow would be up to 3° C. higher, which would make conditions more favourable, and so it is concluded that the increased disease incidence in such sites is due to the higher daily maximum temperatures, and not to the decrease in night temperatures.

A similar explanation can be advanced for the generally observed increase of disease on westerly slopes at the higher altitudes. Kirkpatrick showed the daily maxima to be consistently higher on such exposures and

cited an increase of 1° C. for 7-degree slope, and of 2–2·5° C. for a 20-degree slope. He considered, however, that the increases are 'often considerably greater'. In the higher altitudes, therefore, temperatures in the coffee bushes on such slopes would be, on many occasions, nearer the optimum, while it is also probable that the periods of exposure to suitable temperatures would be prolonged.

### *Effect of shade on disease incidence*

The effect of shade on the incidence of the disease, a subject of great practical importance, has given rise to widely divergent views (Anon., 1934; Rayner, 1952); some observers believe that the disease is worse under shade, others that shade tends to decrease it. Both of these views, depending on the altitude at which the observations were made, may be correct.

Kirkpatrick (1935) showed that in a shaded coffee bush the maximum daily temperatures are 4·5° C. (exceptionally 6·5° C.) lower than in an unshaded one. Shade also reduces the diurnal temperature range by an average of 6·5° C. (exceptionally 11·5° C.). In the lower altitudes, therefore, this effect not only reduces the maximum, but modifies the slope of the time/temperature curve, and permits of longer periods of suitable temperatures on most days, frequently for long enough for invasion to occur. The border-line plantation belonging to District III in Table 3 (which district normally escapes the disease) is, in part, shaded; in these shaded parts the disease occurs at a noticeable, though not economically serious, level (J. B. D. Robinson: private communication). In the lower, and particularly the border-line districts, the presence of shade theoretically can, and, in fact, does increase disease incidence. At the higher altitudes, on the other hand, the effct of shade is to reduce the period during which temperatures remain favourable, and also to reduce the daily maxima, which are rarely, in any case, very much above the optimum. Both of these effects would tend to reduce the incidence of disease. Shade, of course, also modifies the humidity conditions, and the periods for which the berries can remain wet, and thus may well exercise an additional effect.

In summing up, the conclusion that relative humidity has little or no influence on disease incidence in the field may seem surprising until it is realized that, when air-temperatures rise to the minimum at which germination can begin, the air is almost invariably too dry for this to take place. The results of the experiments reported here, taken in conjunction with climatological data, lead us to conclusions which are in direct contrast to the views of Rayner (1952), who postulated that 'whilst climate does play an important role in determining the distribution of the disease, the physiology of the coffee bush may be the key factor and in part climate may work through its effect on this rather than directly'. We, on the other hand, consider that the relationships of the disease with altitude, with rainfall, with shade, and with topography are all readily explicable on the experimental facts concerning conidial production, germination, growth, and penetration and their relation to time, temperature, and humidity.

We wish to acknowledge the courtesy of the Coffee Research Station, Ruiru, for having given us access to certain thermograph and rainfall data in respect of two districts.

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# FUNGAL PARASITES OF THE PHYTOPLANKTON V. *CHYTRIDIUM ISTHMIOPHILUM* SP.NOV.*

By HILDA M. CANTER

*Honorary Mycologist, Freshwater Biological Association, Ambleside, England*

(With 1 Text-figure)

The life-history of *Chytridium isthmiophilum* sp.nov., which parasitizes *Staurodesmus curvatus* (West) Teiling in the plankton of Windermere and Ullswater in the English Lake District, is described.

*Chytridium isthmiophilum* sp.nov. has appeared nearly every year from 1948 to 1959 at some time between July and early December in the north and south basins of Windermere. In Table 1 are records of its occurrence in samples observed at weekly intervals throughout the 12 years with the exception of 23 July–13 Aug. 1951 and 27 July–7 Sept. 1953. It was also found in Ullswater during Aug. 1950 and 1952. Though several other desmids are present in these lakes the chytrid only infects *Staurodesmus curvatus*.

**Chytridium isthmiophilum** sp.nov.

Thallus monocentricus, e sporangio epibiotico per amplificationem zoosporae encystatae effecto, atque ex apophyse endobiotica spherica sine rhizoideis ut excrescentia ex extremitate distali fili germinalis evoluta constans. Sporangium apophysisque simul amplificantur. Apophysis 6–10$\mu$ diam. Sporangium conoideum ad ovatum, 9–30$\mu$ alt. × 5–19$\mu$ lat. media in regione, 10–100+ zoosporas continens. Zoosporae 3$\mu$ diam. globulum magnum sine colore atque flagellum 22$\mu$ long. habentes; in fasciculo immobili, post separationem operculi apicalis 3·5–11·5$\mu$ diam., iam formatae emergentes. Membrana sporangii vacui persistens. Spora quiescens endobiotica, ex inflatione tubi germinalis formata, spherica ad ovatam, 7–15$\mu$ diam., globulos unicum magnum atque paucos minores continens, membrana levi sine colore. Spora quiescens post copulationem terminalem duorum gametarum isogamicorum, 3$\mu$ diam. sexualiter formata, ? uno gameta paulo ante quieto germinatoque.

Thallus in desmidio cognato Staurodesmo curvato (West) Teil. parasiticus, in plancto lacus Windermere dicti (loco scilicet typi) atque lacus Ullswater dicti in regione lacustri Anglica. Typus Herb. I.M.I. no. 83245.

*Thallus* monocentric, consisting of an epibiotic sporangium developed by enlargement of the encysted zoospore and an endobiotic spherical apophysis, without rhizoids, developed as an outgrowth from the distal end of the germ thread. Enlargement of the sporangium and apophysis is simultaneous. Apophysis 6–10$\mu$ diam. *Sporangium* top-shaped to ovoid, 9–30$\mu$ high × 5–19$\mu$ broad in the mid-region and containing from 10 to 100+ zoospores. *Zoospores* 3$\mu$ diam. with a large colourless globule and flagellum 22$\mu$ long; emerging fully formed in a non-motile cluster after detachment of an apical operculum 3·5–11·5$\mu$ diam. Wall of empty

* Studies on British Chytrids. XVI.

sporangium persistent. *Resting spore* endobiotic, formed from a swelling of the germ tube; spherical to oval 7–15 $\mu$ diam. containing a single large and a few smaller globules; wall smooth and colourless. Sexually formed by terminal fusion of two isogamous gametes 3 $\mu$ diam., ? one of which had previously come to rest and germinated.

Parasitic on *Staurodesmus curvatus* (West) Teil. in the plankton of Windermere (type locality; type specimen Herb. I.M.I. no. 83245) and Ullswater in the English Lake District.

The description of the life-history is based on observations made on living material from Windermere. In the discussion that follows it, references are cited only for species since Sparrow (1943).

Table 1. *Occurrence of* Chytridium isthmiophilum *on* Staurodesmus curvatus *from* 1948 *to* 1959

| Year | Windermere (south basin) | Windermere (north basin) |
|---|---|---|
| 1948 | 14 Sept.–10 Nov. | 20 Sept.–22 Nov. |
| 1949 | 18 July–23 Aug., 19 Sept., 3 Oct.–15 Nov. | 18 July–23 Aug., 26 Sept.–28 Nov. |
| 1950 | 2 July | 17 July |
| 1951 | 24 Sept., 15 Oct. | 27 Aug. |
| 1952 | 5 Aug.–18 Sept. | 11 Aug.–6 Oct. |
| 1953 | 20, 27 July, 3 Nov. | 20, 27 July |
| 1954 | 4–18 Oct., 1, 15 Nov. | No record |
| 1955 | 5, 19 Sept. | 5, 19 Sept. |
| 1956 | 13, 20 Aug., 3, 10 Sept., 1 Oct., 5 Nov., 4 Dec. | 30 July, 20 Aug., 3, 10, 24 Sept., 1, 29 Oct., 12, 19 Nov. |
| 1957 | 12, 19 Aug., 30 Sept., 14 Oct.–18 Nov. | 7 Oct.–18 Nov. |
| 1958 | 29 July, 18, 26 Aug., 2, 9 Sept., 21 Oct., 4 Nov., 9 Dec. | 12 Aug.–2 Sept., 14 Oct. |
| 1959 | 7–21 July, 18 Aug. | 30 June–21 July, 18 Aug.–1 Sept. |

The zoospore traverses the mucilage surrounding the desmid (up to 45 $\mu$ wide) and encysts directly on its wall in the region of the isthmus. No zoospore has been observed in the act of passing through the mucilage, and so its method of penetration remains unknown. From the zoospore a thread-like germ tube grows into one of the semi-cells and at its tip a swelling develops. This enlarges into a spherical apophysis, devoid of rhizoids, simultaneously with the expansion of the zoospore cyst into the sporangium. In young stages, owing to the dense algal content, it is often impossible to discern the thread connecting the zoospore with the apophysis and the wall of the apophysis itself. However, from an early stage the apophysis usually contains a single large refractive globule which makes its position in the desmid easily locatable (Fig. 1 *a*, *b*). The sporangium is top shaped to ovoid (Fig. 1 *h–j*) and occasionally borne on a short slightly thickened extramatrical stalk (Fig. 1 *g*(*X*)). At maturity the apical portion, which will be detached as the operculum, is clearly visible. Changes in the cytoplasm leading to the formation of zoospores are similar to those described for the majority of chytrids. On dehiscence the zoospores, each with a flagellum coiled round its body, emerge in a non-motile cluster. The operculum either remains at the side of the sporangium or is carried up on top of the zoospore mass. The zoospores at the edge very soon jerk their flagella and free themselves. No vesicle surrounding the zoospores

Fig. 1. *Chytridium isthmiophilum* sp.nov. *a–f*, stages in growth of sporangium and apophysis; *g*, sporangium freed from its host by squashing [(*X*), thickened extramatrical stalk-like portion, (*Y*) endobiotic part of germ thread, (*Z*) apophysis]; *h–j*, mature sporangia; *k*, zoospore; *l*, *m*, empty sporangia; *n*, fused isogamous gametes, the resting spore is in such a position that the endobiotic portion of the germ thread connecting it with the gametes cannot be seen; *o*, gametes of (*n*) at a higher magnification; *p*, *q*, *r*, immature resting spores; *s*, mature resting spore; *t*, resting spore in surface view showing the encrusted reddish granules. *n*, × 875; *o*, × 1125; rest × 650.

has been observed. When swimming, the body of the zoospore itself seems to vibrate and the refractive globule, somewhat anterior in position, becomes difficult to distinguish. The wall of the empty sporangium is persistent.

As the fungus develops the chloroplast of the desmid shrinks, at first retaining its green colour, but finally only a reddish brown residue remains.

The resting spore is endobiotic in position and formed by enlargement of the germ thread. That a sexual process is involved is seen in Fig. 1*n*, *o*. Two bodies resembling encysted zoospores were often seen at the isthmus of a desmid containing a resting spore but proof that they are gametes was lacking until 1959 when the stage figured above was found. Whether one gamete comes to rest and germinates before the other fuses with it is unknown. Commonly a granule or globule remains in the body of the gamete after the resting spore is formed. The resting spore is spherical to subspherical and connected to the gametes by a slender portion of the germ thread. During development, the content consists of numerous globules (Fig. 1*p–r*) but at maturity it is dominated by a single large one (Fig. 1*s*). The wall is smooth and colourless, often with an external deposit of red granular material (Fig. 1*t*), probably the remains of the desmid chloroplast. Germination of the resting spore has not been seen.

Within the genus *Chytridium* several species resemble *C. isthmiophilum* by the possession of an endobiotic apophysis devoid of rhizoids, namely, *C. brevipes* Braun, *C. cejpii* Fott (1951). *C. chlorobotrytis* Fott (1952), *C. inflatum* Sparrow, *C. mallomonadis* Fott (1957), *C. scherffelii* Sparrow and *C. sexuale* Koch (1951). Similarly, they all have a sporangium formed by enlargement of the zoospore and apparently growth of the apophysis and sporangium is simultaneous. Endobiotic resting spores arising as a swelling of the germ tube are described for *C. isthmiophilum*, *C. chlorobotrytis* and *C. sexuale*. In *C. brevipes* the resting spore is described as endobiotic, but differs from those above since it is formed within the equivalent of the sporangium. Whether this is indeed the true resting spore of *C. brevipes* is questionable. It may well be the resting spore of a hyperparasite such as *Rozella*. *C. chlorobotrytis* has an asexually formed resting spore, whereas in *C. sexuale* and *C. isthmiophilum* it is sexual. In *C. sexuale* the fusing gametes are anisogamous while in *C. isthmiophilum* they are isogamous.

Koch (1951, fig. 2) showed that the size of the resting spore protoplast in *C. sexuale* lay outside the range exhibited by the apophysis. Whereas in the other species with which he compared it, i.e. *C. oedogonii* Couch, *C. schenkii* (Dang.) Scherffel, *C. aggregatum* Karling, *C. stellatum* (Petersen) Koch (1951) and *C. lagenaria* Schenk pro parte, the dimensions of the resting spore protoplast lay within those of the apophysis. Since *C. sexuale* was the only species with a sexually formed resting spore Koch suggested that the separation of size ranges might be related to sexuality. It must be noted that Koch compared *C. sexuale* with species exhibiting an endo-exogenous method of development (Karling, 1936) and in which the apophysis bears rhizoids. By the description of *C. chlorobotrytis* and *C. isthmiophilum* it is now possible to compare *C. sexuale* with species which by their method of development and morphology appear more closely related to it. In *C. isthmiophilum* the dimensions of the sexually formed

resting spore exceed those of the apophysis, but unlike *C. sexuale* do not lie completely outside the size range of it. On the other hand, in *C. chlorobotrytis*, judging from Fott's illustrations (1952; fig. 4*a*, *b*, *d–f*), measurements are only given for the apophysis, the size of the resting spore though asexual appearing to lie outside the range of the apophysis. The significance of such comparisons is open to doubt as the size differences involved are often no more than a few microns and further observations would probably alter the size ranges.

However, from the above discussion it is evident that within the genus *Chytridium* there are at present two groups of species, living on algae, which have in common an epibiotic sporangium and an endobiotic apophysis and resting spore. In the first group the apophysis is devoid of rhizoids and development of it and the sporangium go hand in hand. Again, from the evidence so far, the resting spore whether sexual or asexual exceeds the size range of the apophysis. In the second group the apophysis bears rhizoids and the thallus development is endo-exogenous. Here only asexual resting spores are known but they never exceed the size range of the apophysis. It could be that the separation of size ranges between the resting spore and apophysis are not related to sexuality but are rather more concerned with the type of thallus development and to the presence or absence of rhizoids.

I acknowledge that the microscope used in this investigation was obtained from the Central Research Fund of the University of London. My thanks are due to Dr Hanna Croasdale, Dartmouth College, U.S.A., for the Latin translations, and especially to Dr J. W. G. Lund for his helpful criticism of the manuscript.

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# STRAINS OF *PHYTOPHTHORA PALMIVORA* (BUTL.) BUTL. FROM *THEOBROMA CACAO* L.

## I. ISOLATES FROM WEST AFRICA

By P. D. TURNER

*West African Cocoa Research Institute, Tafo, Ghana*

(With Plate 27 and 1 Text-figure)

Distinct strains of *Phytophthora palmivora* were found when 179 isolates from ten cocoa-producing countries in West Africa were examined. Comparative tests showed the strains to differ in lesion development on cacao pods, in sporangial dimensions and in geographical distribution. Results of nearly 900 crossing tests showed that the two dominant strains were sexually complementary, whilst another small group of isolates, atypical of *P. palmivora*, was complementary to neither of the other two. Classification and distribution of the strains are discussed.

*Phytophthora palmivora*, the causal fungus of black pod disease and other infections of *Theobroma cacao* L., has been recorded on cacao in all the countries in West Africa where this crop is grown. It has remained unknown, however, whether the pathogen is identical throughout the region, although marked differences between cacao isolates from Ghana and Nigeria were reported by Wharton (1958). As a result of these observations, isolates have been collected from all the cocoa-producing territories in West Africa with the exceptions of Liberia, Togo, Rio Muni, Central African Republic and São Thomé. Altogether, 179 isolates from the following countries have been examined: Sierra Leone, 6; Ivory Coast, 7; Ghana, 57; Nigeria, 47; Southern Cameroon, 19; Fernando Po, 7; Cameroun, 30; Gabon, 5; and Belgian Congo and Angola one isolate each. The main comparative characters used were those of development in cacao pods, sporangial dimensions and oospore production. Evidence for the existence and regional distribution of different strains of *P. palmivora* in West Africa is summarized in this paper.

### Growth in Cacao pods

Wherever possible, diseased pods or infected vegetative material were collected from numerous and widespread localities within each country. Pieces of diseased leaves and chupons were first inserted into healthy pods and the fungus isolated, from the edges of the lesions which subsequently developed, by inoculation on to 4% oatmeal agar. Inoculation of pods in the field was precluded by phytosanitary precautions. For comparative growth tests, inoculations into detached, unripe pods of the same variety and approximate age were made, in replicates of ten, using similar inocula from the margins of fresh lesions in artificially infected pods. Inoculation

of each isolate into the test pods was standardized by removing a tissue disk from the centre of each pod with a 3·5 mm. diam. cork borer, and filling the cavity with an equal-sized piece of infected tissue. The inoculated pods were incubated in humid chambers made of polythene sheeting stretched over wire frames, at an ambient temperature of approximately 80–85° F. The size of lesions produced by all isolates was compared with that of the typical isolate from cacao pods at Tafo. Measurements of the length plus diameter of lesions were made after 5 days, and the data analysed using Student's '*t*' test.

Lesions in unripe pods were of three kinds; the distinction in ripe pods was definite but less pronounced. The first type of lesion was characterized by a well-defined and regular margin, with sparse aerial mycelium, and clumps of sporangia that were scattered and discrete. Lesions of the second type often had a lighter brown coloration, with margins which were diffuse and irregular; there was a negligible amount of aerial mycelium, but dense, confluent spore masses gave the lesion a powdery appearance. The third, least common, type of lesion had sharply delimited margins, but such sporangia and surface mycelium as were produced did not develop until at least 7 days after inoculation and were, even then, extremely sparse. Isolates from Ivory Coast, Ghana, Belgian Congo and the majority from Sierra Leone all produced lesions of the first type, whereas almost all isolates from Nigeria, Southern Cameroon, Cameroun, Fernando Po and Gabon were all of the second type. Isolates of the first category were also recorded from two areas in Nigeria and once from the Southern Cameroon. The single isolate from Angola and two from Sierra Leone produced lesions of the third variety. As the distribution based on lesion appearance also applied to sporangial dimensions and oospore production described below, the categories are, for convenience, referred to as the Ghanaian (G), Nigerian (N) and Angolan (A) types respectively (Plate 27).

Lesions produced by isolates of the G type usually expanded faster than those of the N and A groups of isolates. Lesion dimensions of representative isolates from each country are summarized in Table 1. Some variation, however, did occur. Three isolates from Ghana grew at consistently lower rates than the normal isolate, but were characteristic of the type in all other respects. Considerable variation was found between isolates of the N type from all countries where it occurred, samples from within the same locality were sometimes inconsistent, and lesion development was often very slow. It was also observed that N type isolates inoculated into ripe pods grew at the same or, more usually, at a greater rate than in unripe pods, whereas the reverse applied with isolates of the G type and variable rates were found with the A type isolates.

### Sporangial characters

The regional differences in size and appearance of sporangia from the groups of isolates were as great as those in pod lesion characteristics. Sporangia of the G type of isolate were of the rather elongated form typical of *P. palmivora*, whereas those of the N type isolates were broader and

Table 1. *Size of lesions (length plus diameter) produced by isolates of* Phytophthora palmivora *in unripe cacao pods*

| | | Isolate type | Lesion size (cm.) | Tafo isolate | Lesion size as % Tafo isolate | *P* |
|---|---|---|---|---|---|---|
| Angola | 1 | A | 9·9 ± 2·0 | 26·0 ± 1·4 | 38 | 0·001 |
| Sierra Leone | 1 | A | 18·6 ± 3·4 | 26·5 ± 1·4 | 70 | 0·001 |
| | 2 | G | 23·5 ± 1·5 | 26·4 ± 2·1 | 89 | NS |
| | 3 | G | 26·8 ± 1·8 | 26·5 ± 1·4 | 101 | NS |
| Ivory Coast | 1 | G | 26·6 ± 1·8 | 26·5 ± 1·8 | 100 | NS |
| | 2 | G | 27·2 ± 1·7 | 26·5 ± 1·8 | 103 | NS |
| Ghana | 1 | G | 23·4 ± 1·0 | 23·7 ± 1·7 | 99 | NS |
| | 2 | G | 24·6 ± 1·8 | 23·7 ± 1·7 | 104 | NS |
| | 3 | G | 23·3 ± 1·8 | 23·7 ± 1·7 | 98 | NS |
| Belgian Congo | 1 | G | 26·4 ± 2·0 | 26·9 ± 2·0 | 98 | NS |
| Nigeria | 1 | G | 24·5 ± 2·2 | 24·4 ± 1·0 | 100 | NS |
| | 2 | G | 24·8 ± 2·2 | 26·5 ± 1·7 | 94 | NS |
| | 3 | N | 17·2 ± 1·3 | 27·2 ± 1·1 | 63 | 0·001 |
| | 4 | N | 13·2 ± 1·4 | 26·5 ± 1·8 | 50 | 0·001 |
| | 5 | N | 19·0 ± 1·3 | 25·1 ± 1·7 | 76 | 0·001 |
| Southern Cameroon | 1 | N | 11·6 ± 0·8 | 27·0 ± 2·0 | 43 | 0·001 |
| | 2 | N | 9·6 ± 1·9 | 27·0 ± 2·0 | 36 | 0·001 |
| | 3 | G | 28·5 ± 1·6 | 26·9 ± 2·0 | 106 | NS |
| Fernando Po | 1 | N | 6·7 ± 1·3 | 25·5 ± 1·5 | 26 | 0·001 |
| | 2 | N | 10·9 ± 1·9 | 25·9 ± 1·3 | 42 | 0·001 |
| Cameroun | 1 | N | 15·7 ± 0·7 | 25·5 ± 1·5 | 62 | 0·001 |
| | 2 | N | 14·6 ± 1·4 | 25·9 ± 1·3 | 56 | 0·001 |
| Gabon | 1 | N | 16·2 ± 1·3 | 26·1 ± 1·5 | 65 | 0·001 |
| | 2 | N | 15·9 ± 1·9 | 25·5 ± 2·4 | 62 | 0·001 |
| | 3 | G | 28·6 ± 1·3 | 27·2 ± 1·1 | 105 | NS |

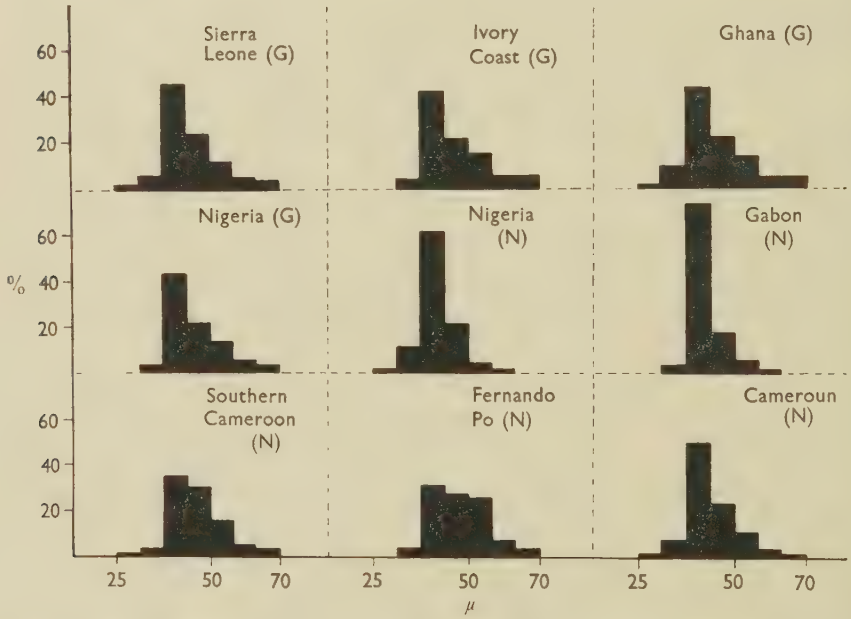
*A* = Angolan, G = Ghanaian, N = Nigerian. NS = no significant difference, $P > 0{\cdot}05$.

Table 2. *Mean sporangial dimensions* ($\mu$) *of* Phytophthora palmivora *isolates*

| | Isolate type | No. isolates measured | Mean length (cm.) | Mean breadth (cm.) | L:B ratio |
|---|---|---|---|---|---|
| Angola | A | 1 | 40·0 | 24·8 | 1·61 |
| Sierra Leone | A | 2 | 42·0 | 24·6 | 1·70 |
| | G | 4 | 42·6 | 25·4 | 1·68 |
| Ivory Coast | G | 7 | 43·6 | 26·5 | 1·65 |
| Ghana | G | 57 | 42·0 | 25·0 | 1·68 |
| Belgian Congo | G | 1 | 46·4 | 26·5 | 1·75 |
| Nigeria | G | 5 | 43·4 | 24·0 | 1·81 |
| | N | 42 | 38·3 | 32·0 | 1·20 |
| Southern Cameroon | G | 1 | 42·6 | 24·9 | 1·71 |
| | N | 18 | 43·1 | 30·0 | 1·44 |
| Gabon | G | 2 | 43·3 | 25·0 | 1·73 |
| | N | 2 | 38·9 | 31·5 | 1·23 |
| Fernando Po | N | 7 | 44·2 | 34·0 | 1·30 |
| Cameroun | N | 30 | 41·2 | 30·0 | 1·37 |

sometimes almost spherical. The more rounded form of sporangium in the latter group also made the apical papillae appear more prominent. Such sporangia as were produced by the A type isolates were of similar size to the G type, but were usually distorted to a greater or less degree. The length and breadth dimensions of 100 sporangia taken after 5–6 days from pods infected with each isolate were measured, and the composite results for each country are shown in Table 2. Sporangia from isolates of the A type were measured as they became available.

Although the range of mean sporangium length for each territory was very similar, the distribution of class boundaries for sporangial lengths was variable, especially in most countries with the N type of pathogen. The distribution of sporangial length class boundaries on a national basis is




Text-fig. 1. Mean distribution of class length boundaries for sporangial length of *P. palmivora* isolates. G = Ghanaian type; N = Nigerian type.

shown in Text-fig. 1. As with pod lesion measurements, greater variation in sporangial dimensions was found between isolates of the N type than between those of the G type. Although Gadd (1924) and Butler (1929) did not consider spore dimensions alone to be reliable taxonomic characters, the lower ratio of sporangial length:breadth observed in this survey is characteristic of the N type of isolate, and would appear to be of some taxonomic value. In the original description of the species (Butler, 1907), the sporangial length:breadth ratio of 1·4 is similar to that of the N group of isolates.

### Oospore production

*P. palmivora* is sometimes considered to be a heterothallic fungus with regard to production of oospores, although homothallic strains have been found (Ramakrishnan & Seethalakshmi, 1956). Gadd (1924) reported oospore production only in paired cultures of the 'cacao' and 'rubber' groups of the fungus. Examination of a number of West African isolates for oospore production, in both single and paired cultures, was made by growing them on 4% oatmeal agar slopes. Ashby (1929) found that oospores were very slow to develop at tropical temperatures, so the cultures were maintained at 60° F. Isolates from widely spaced localities within each country were grown together, and cultures of each fungus type from all countries were intercrossed. The results of 890 tests for oospore production, examined after 3 weeks' growth, are summarized in Table 3.

Table 3. *Oospore production by isolates of* Phytophthora palmivora

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ghana (G) | O | Ghana (G) | | | | | | | | | | | | | |
| Sierra Leone (G) | O | O | Sierra Leone (G) | | | | | | | | | | | | |
| Ivory Coast (G) | O | O | O | Ivory Coast (G) | | | | | | | | | | | |
| Belgian Congo (G) | O | O | O | O | Belgian Congo (G) | | | | | | | | | | |
| Southern Cameroon (G) | O | O | O | O | O | Southern Cameroon (G) | | | | | | | | | |
| Gabon (G) | O | O | O | O | O | O | Gabon (G) | | | | | | | | |
| Nigeria (G) | O | O | O | O | O | O | O | Nigeria (G) | | | | | | | |
| Nigeria (N) | X | X | X | X | X | X | X | O | Nigeria (N) | | | | | | |
| Southern Cameroon (N) | X | X | X | X | X | X | X | O | O | Southern Cameroon (N) | | | | | |
| Fernando Po (N) | X | X | X | X | X | X | X | O | O | O | Fernando Po (N) | | | | |
| Cameroun (N) | X | X | X | X | X | X | X | Ox | Ox | O | O | Cameroun (N) | | | |
| Gabon (N) | X | X | X | X | X | X | X | O | O | O | O | O | Gabon (N) | | |
| Angola (A) | O | O | O | O | O | O | O | O | O | O | O | O | O | Angola (A) | |
| Sierra Leone (A) | O | O | O | O | O | O | O | O | O | O | O | O | O | O | Sierra Leone (A) |

X = oospores produced in mixed culture. O = no oospores produced. Ox = oospores produced in one crossing test only. A = Angolan, G = Ghanaian, N = Nigerian type.

The results confirm and emphasize the distinction between the three groups of isolates. No oospores were found in paired cultures of the G type of fungus, either within or between countries. Oospores were only occasionally found in crossings between the N type isolates. No oospores were found in any crosses with isolates of the A type. However, all paired cultures of the G and N types produced oospores. When oospores were produced they were normally found in abundance, and occurred throughout the entire culture. The antheridia were amphigynous, and the oospores ranged from 16·7 to 25 (av. 20·3) $\mu$ diam., with an oogonial wall of 31 $\mu$ av. diam. Their origin was from two different hyphae but it was not possible to trace the hyphae to their parent isolates. No attempt was made to germinate the oospores.

### Identity of the isolates

The origin of *P. palmivora* in West Africa is unknown, but the ability of the fungus to attack members of fifty-one host genera (Hickman, 1958), of which several are indigenous to Africa, suggests that the pathogen was

almost certainly present before cacao was introduced. In the Ivory Coast Renaud (1957) obtained a culture of *P. palmivora* from *Cola nitida* which was less pathogenic than isolates from cacao, and occasional isolates of lower pathogenicity have also been found in Ghana (Wharton, 1959). Robertson (1947) described isolates from rubber seedlings in Ghana which were indistinguishable from the cacao pathogen, and attempts by the author to distinguish between isolates from cacao, rubber pods, *Herrania* sp., and *Gmelina arborea*, including tests for oospore production, have similarly failed (Turner, 1960). Dade (1928), also in Ghana, obtained an isolate of *P. palmivora* from *Mimusops elengi* which was distinctly different from the cacao isolate, and which produced oospores in mixed culture with the latter. Photographs and records show that the sporangial shape and dimensions and the form of pod lesions closely resembled those of the N type of isolate.

Demonstration that the N type of isolate is sexually complementary to the G type indicates that the N type should be placed in the 'rubber' group of *P. palmivora*. In addition, oospores were also produced when isolates of the N type were grown in mixed culture with a 30-year-old isolate of the 'cacao' group from *Cocos nucifera*. Rubber is extensively grown in Nigeria and Southern Cameroons, but isolates of the N type are unlike isolates examined from rubber from Malaya and Ceylon. However, the differences found in pod lesion development, sporangial dimensions and occasional oospore production between isolates illustrate the heterogeneity and complexity of the N group as a whole, and it may well be that the group can be subdivided into component strains or races.

Isolates of the A type show fundamental differences from the other groups, particularly in cultural characteristics and in failure to produce oospores with either group. They very closely resemble an isolate from rubber received from Malaya; this produces an identical form of pod lesion and has also failed to produce oospores in crossing tests with all other isolates. The A type would appear to belong to neither the 'cacao' nor the 'rubber' groups of *P. palmivora*, from the evidence of oospore production, but resembles the 'atypical' form of the species in cultural characters.

## Discussion

Further dissimilarities between the isolates have been revealed by comparison of characters in artificial culture, but data from the three types of comparative test—physiological, morphological and sexual—were sufficient to demonstrate that distinct and complementary strains of *P. palmivora* occur on cacao in West Africa, and that distribution of the strains substantially follows a definite geographical pattern. In the three more westerly cocoa-producing countries of Sierra Leone, Ivory Coast and Ghana, with rare exceptions, only one type of the pathogen has been found on cacao, and this type also occurred sporadically in other countries from which isolates have been examined. The dominant pathogen in the easterly countries of Nigeria, Southern Cameroon, Cameroun and Fernando Po differed in several distinct characters. The precise position of Gabon, Belgian Congo and Angola is obscure; the dominant pathogen

in Gabon is probably of the N type, but further isolates would be required before the countries could be typified. The basis of the differences is unknown.

Similarity between isolates from Ghana and Ivory Coast would be expected owing to the almost continuous cacao-growing zone between the two countries. A similar situation occurs across the border between Ghana and Togo, and the G type of isolate might be anticipated to be the only or at least the dominant type in Togo. It is possible that the distance of about 150 miles between the eastern edge of the cacao-growing area in Togo and the western limit of cacao in Nigeria provides a natural barrier to widespread dispersal of the different fungal types. On the other hand, the evidence suggests that the G type of isolate may be dominant in the westerly countries through greater virulence. It may be significant that, after a lapse of 30 years, isolates collected from cacao, from the site adjacent to the infected *Mimusops elengi* mentioned above, were all of the G type, with no apparent spread of the 'rubber' group pathogen, although in earlier days the *Mimusops elengi* had always been heavily infected (Dade, personal communication). The distribution of the G type of isolate from Ghana to other West African territories might well have been effected through the export of hundreds of thousands of cacao pods from Ghana as planting material, which has been in progress for many years; some of these pods would undoubtedly have carried latent infections of *P. palmivora*.

The occurrence of complementary isolates within the same area would result in genetic variation, including that in pathogenicity, if both strains participate in oospore formation, and the presence of the G type in the N group of countries could partly explain the variation found, though natural production of oospores in the field has never been recorded.

Existence of different strains of a pathogen within a territory is particularly important as the crossing of complementary strains may constitute a source of new types varying in pathogenicity that could be of danger when new, potentially disease-resistant, types of plant are produced. There is already a good prospect of producing types of cacao resistant to *P. palmivora* in Ghana (Wharton, 1960), and it is obvious that such types will need more extensive testing in regions other than Ghana. Variability in the pathogen may also complicate the results of experiments on chemical control of disease.

I am indebted to the many people who have either sent material or provided hospitality during collecting visits; to Dr J. W. Blencowe and Mr A. L. Wharton for helpful criticism; and to the staff of the Pathology Division of this Institute for technical assistance.

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## EXPLANATION OF PLATE 27

A, B. Lesions of the Ghanaian type produced by isolates from Ghana (A) and Sierra Leone (B) after 5 days.

C, D. Lesions of the Nigerian type produced by isolates from Cameroun (C) and Southern Cameroon (D) after 7 days.

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(*Facing p.* 672)

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# LONGEVITY OF CONIDIA OF *BOTRYTIS FABAE* SARDIÑA

By F. T. LAST

*Rothamsted Experimental Station, Harpenden, Herts*

(With 2 Text-figures)

The numbers of conidia produced by *Botrytis fabae* on agar media increased up to the tenth day after inoculation, when the average age of conidia was 3 days. Germination in water of conidia from cultures differing in age between 10 and 40 days was equally high but those from cultures 25 and 35 days old were only 1/10 and 1/100 as infective as conidia from 10-day-old cultures.

Suspending conidia from cultures up to 40 days old in a solution (0·2 % or higher concn.) of either yeast extract or orange juice partially restored infectivity, as did solutions of sucrose, glucose, mannose and maltose, but fructose and galactose were less effective. Arabinose, xylose, casein hydrolysate, peptone and nucleic acid did not increase infectivity.

Abrading leaves with 'Celite' before inoculation increased the numbers of infections produced by spores suspended in water; the increase, as with adding sugars, was greater with old than with young conidia.

The longevity of spores has usually been assessed by their ability to germinate (Hawker, 1950; Cochrane, 1958). To reproduce, however, most fungi must be able not only to germinate, but also to colonize suitable substrates. When conidia of some plant pathogens, e.g. *Erysiphe graminis* DC. and *Botrytis fabae* Sardiña, are exposed to ultraviolet radiation, they lose the power to infect more rapidly than the ability to germinate (Buxton, Last & Nour, 1957). A similar effect with infectivity lost more rapidly than the ability to germinate occurs when cultures of *B. fabae* age and is described in this paper.

## Materials and methods

An isolate of *B. fabae* (I.M.I. Herb. no. 79681) which readily infects leaves of broad beans cv. Seville Longpod, was grown at 20° C. on an agar medium containing (g./l. $H_2O$): $KH_2PO_4$, 1·52; $MgSO_4$ $7H_2O$, 0·52; $NaNO_3$, 6·0; KCl, 0·52; dextrose, 10·0; peptone, 2·0; casein hydrolysate, 3·0; yeast nucleic acids, 0·5; agar, 20·0. On this medium the numbers of conidia per unit area of colony increased up to the tenth day, when their average age was 3·1 days (Fig. 1). Subsequently their numbers (*ca.* $5{\cdot}8 \times 10^5$/cm.$^2$) did not differ significantly, but from the fifteenth day onwards a few conidia were found already germinating and producing numerous microspores (see Brierley, 1918, for review of microspore formation).

Conidia were washed from cultures with distilled water, separated from unwanted mycelium by filtering through cotton-wool and then washed five more times. Their ability to germinate in water at 20° C. (the optimum)

was tested in Böttcher's cavity slides (diam. *ca.* 1·5 cm.) containing 0·3 ml. conidial suspension, following the standards set by the American Phytopathological Society (1943), excepting that orange juice (a 'stimulant') was omitted. Suspensions with about $3 \times 10^3$ conidia/ml. were used and incubated in saturated atmospheres. At concentrations of $10^5$ or more, the percentage germination decreased, especially with conidia from old cultures, e.g. increasing numbers from $4 \times 10^4$ to $4 \times 10^5$/ml. decreased the germination of conidia from cultures 7 days old from 91 to 68% and of conidia from cultures 18 days old from 91 to 20%.

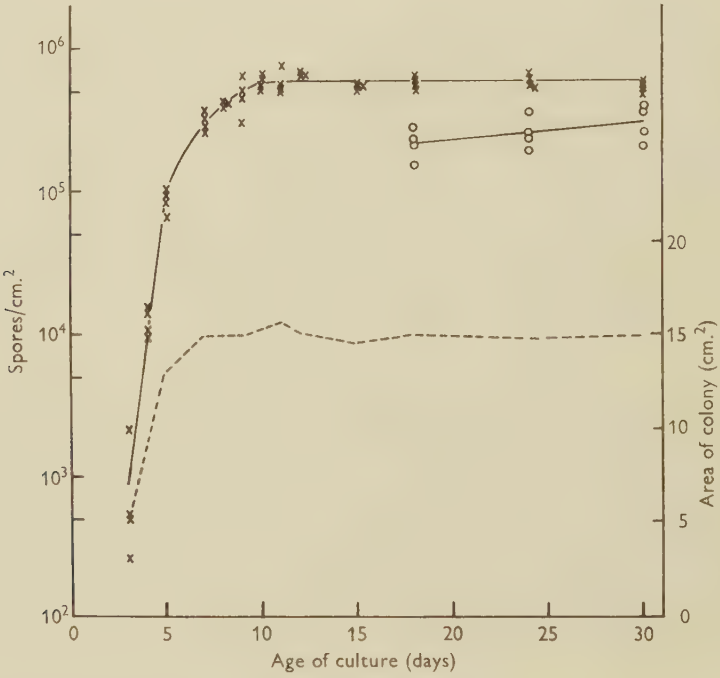


Fig. 1. The size of colony and production of conidia (×) and microspores (○) by cultures of *Botrytis fabae* at different intervals after inoculation (broken line indicates area of colony; spores/cm.² are plotted on log scale).

Infectivity, again at *ca.* 20° C., was tested by rubbing broad bean leaves with conidia suspended in water, incubating in a saturated atmosphere for 48 hr. and then counting numbers of lesions/½ leaflet (Last & Hamley, 1956). For statistical analysis these numbers ($n$) were transformed to $z$, where

$$z = \log_{10} \tfrac{1}{2}[x + c + \sqrt{(x^2 + 2cx)}]$$

and $c = 20$ (see tables in Kleczkowski, 1955). Because numbers of lesions are not simply related to the concentrations of inoculum, it was necessary to calculate the numbers of active conidia in suspensions of different ages,

before determining relative infectivities. The numbers of active conidia were obtained, assuming conidia from cultures 7–10 days old were fully infective, from

$$Y = mX + c,$$

where $X$ and $Y$ are logarithms of the inoculum concentration and lesion number per $\frac{1}{2}$ leaflet respectively, $m$ is the slope which averaged 0·64 in five similar dilution series tests, and $c$ is a constant.

Brierley (1918) found that microspores germinated readily, but those produced by isolates used in the present experiments did not. Because microspores do not affect the numbers of lesions given by conidial suspensions (Last & Hamley, 1956) the mixed suspensions were left unseparated.

## Results

Fig. 2 shows the relative abilities of conidia taken from cultures of different ages to germinate in distilled water and to infect broad bean leaves. The results are means of at least seven experiments and are arranged according to age of culture 0–10 days, 11–20 days. Although equal percentages of conidia from 10- and 40-day-old cultures germinated (relative value of 100 % is equivalent to an absolute value of 93 % germination), only those from the younger culture infected beans. Whereas infectivity fell by 90 % within 25 days of culturing, germination did not fall to this extent until 85 days (Fig. 2).

Much is known of the nutritional requirements for germination of a wide species range of fungus spores (Hawker, 1950; Cochrane, 1958), but requirements for infection have been little studied. Ageing conidia of *B. fabae* seem to contain adequate reserves for germination but insufficient to meet the demands for infection. Tests were therefore made to see whether infectivity could be restored to conidia from cultures 20–30 days old by supplementing their nutrition.

Of plant juices known to increase the vigour of germination (Gottlieb, 1950), orange juice is commonly added when testing fungicides. Undiluted orange juice, in place of distilled water, significantly increased numbers of lesions produced by conidia from cultures 20–30 days old (Table 2). Orange juice contains, apart from proteins and vitamins, 1·5 % citric acid and 4·5 % sucrose (Mottram & Graham, 1941). In further tests, the effect of orange juice on infectivity was paralleled by 4·5 % sucrose, which suggests that the conidia lacked a carbon source rather than micronutrients, e.g. vitamins. This was again found in tests using yeast extract, which at low concentrations did not increase numbers of lesions but did so at concentrations of 0·2–5·0 %.

The effect of orange juice seems to be mainly attributable to sucrose which, like yeast and orange juice, needs to be used at a concentration of, at least, 0·2 % to increase infectivity. At a concentration of 4·5 %, citric acid and sucrose increased the numbers of lesions from 9·5 in the water controls to 21·0 and 35·5 per $\frac{1}{2}$ leaflet respectively. In tests with a range of sugars, results varied between experiments but sucrose consistently increased infectivity the most, followed by glucose, mannose and maltose; the pentose sugars, arabinose and xylose, had no effect (Table 2).

The infectivity of conidia from young cultures, 13 and 19 days old, was not significantly increased by galactose, but the numbers of lesions produced by cultures 33 and 41 days old were increased by factors of $\times 5{\cdot}0$ and $\times 3{\cdot}6$, respectively. Similarly, sucrose increased the infectivity of old cultures more than that of young cultures; numbers of lesions given by conidia from cultures 5, 24 and 35 days old were increased by $\times 1{\cdot}7$, $\times 6{\cdot}0$ and $\times 4{\cdot}3$, respectively (Table 3).

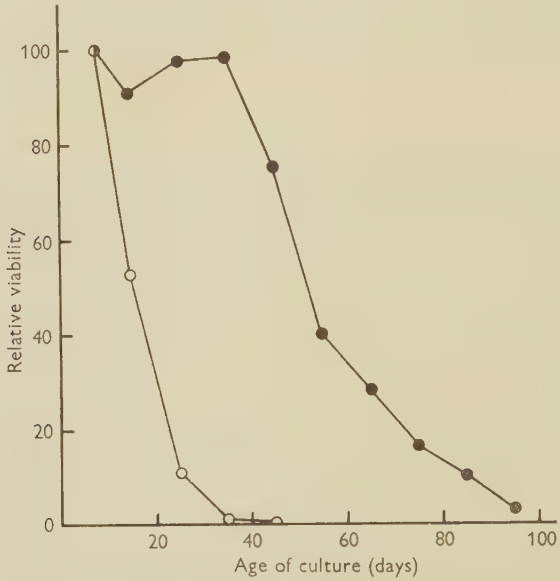


Fig. 2. The relative abilities of conidia of *Botrytis fabae* taken from cultures of different ages to germinate (●) and to infect (○) broad bean leaves at *ca.* 20° C.

Table 1. *Effect of orange juice and some of its components on numbers of lesions/$\frac{1}{2}$ leaflet given by* Botrytis fabae *conidia from cultures about 25 days old (transformed data in bold)*

| Expt. | Water | 1·5% citric acid | 4·5% sucrose | Undiluted orange juice | L.S.D. ($P = 0{\cdot}05$) |
|---|---|---|---|---|---|
| A | 6 | 18 | 70 | 39 | |
| | **1·33** | **1·54** | **1·95** | **1·76** | **0·200** |
| B | 4 | 7 | 47 | 33 | |
| | **1·26** | **1·35** | **1·82** | **1·71** | **0·182** |

Effects similar to those given by favourable sugars were obtained by abrading leaves with dry 'Celite', a diatomaceous earth, before inoculating with water suspensions. Celite usually increased numbers of lesions, but the size of the effect depended on age of the culture (Table 4). It had no effect on lesions given by cultures 107 days old; it increased the numbers given by very young cultures, *ca.* 10 days old, by a factor of up to $\times 4$, and of intermediate cultures, ranging from 19 to 48 days, by at least $\times 16$.

Table 2. *Numbers of lesions developing per* $\frac{1}{2}$ *leaflet when various sugars* (1 %) *were added to suspensions of* Botrytis fabae *conidia taken from cultures at least* 20 *days old* (*transformed data in bold*)

| Expt. | Water | *l*-Arabinose | *d*-Arabinose | Xylose | Lactose | Raffinose | Galactose | Fructose | Maltose | Mannose | Glucose | Sucrose | L.S.D. ($P = 0.05$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | 3<br>**1·22** | 2<br>**1·20** | 2<br>**1·18** | — | — | 7<br>**1·35** | 5<br>**1·29** | 8<br>**1·38** | — | 15<br>**1·50** | — | 19<br>**1·56** | **0·093** |
| 25 | 2<br>**1·21** | 1<br>**1·14** | — | — | — | 3<br>**1·25** | 2<br>**1·20** | 3<br>**1·23** | — | 4<br>**1·28** | 4<br>**1·27** | 9<br>**1·40** | **0·112** |
| 32 | 19<br>**1·56** | 18<br>**1·55** | — | — | — | 18<br>**1·55** | 19<br>**1·56** | 42<br>**1·78** | — | 42<br>**1·78** | 66·5<br>**1·93** | 64·5<br>**1·92** | **0·104** |
| 33 | 2·5<br>**1·22** | — | — | 1<br>**1·15** | 3<br>**1·24** | — | 12<br>**1·46** | 5<br>**1·29** | 5<br>**1·31** | — | 10·5<br>**1·43** | 9·5<br>**1·41** | **0·135** |
| 35 | 13·5<br>**1·48** | — | — | 14<br>**1·49** | 25<br>**1·63** | — | 27<br>**1·65** | 30<br>**1·68** | 29<br>**1·67** | — | 43<br>**1·79** | 42<br>**1·78** | **0·119** |
| 36 | 8<br>**1·38** | — | — | 12<br>**1·46** | 12<br>**1·46** | 13<br>**1·47** | — | — | 32<br>**1·70** | 46<br>**1·81** | 20·5<br>**1·58** | 47·5<br>**1·82** | **0·126** |

Although the infectivity of equal concentrations of conidia from cultures 11 and 19 days old differed significantly without 'Celite', giving forty-nine and eight lesions respectively, with 'Celite' both gave about 200 lesions.

Adding casein hydrolysate (a source of amino acids), peptone and nucleic acid (sodium salt from yeast) at concentrations of 1 % did not increase the infectivity of conidial suspensions.

Table 3. *Increase in numbers of lesions/½ leaflet produced by adding sugars* (1 %) *to suspensions of* Botrytis fabae *conidia taken from cultures of different ages* (*transformed data in bold*)

| | Age of culture (days) | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | 5 | 13 | 19 | 24 | 33 | 35 | 41 | (±0·020) |
| Expt. III (±0·035) | | | | | | | | |
| Sucrose | 103 | — | — | 81 | — | 17 | — | |
| | **2·09** | | | **2·00** | | **1·53** | | **1·87** |
| No Sucrose | 61 | — | — | 14 | — | 4 | — | |
| | **1·90** | | | **1·48** | | **1·28** | | **1·55** |
| Expt. IV (±0·086) | | | | | | | | Mean |
| Galactose | — | 128 | 119 | — | 54 | — | 29 | (±0·043) |
| | | **2·14** | **2·11** | | **1·86** | | **1·67** | **1·95** |
| No Galactose | — | 135 | 88 | — | 11 | — | 8 | |
| | | **2·16** | **2·03** | | **1·44** | | **1·37** | **1·75** |

Table 4. *Numbers of lesions/½ leaflet produced by conidia of* Botrytis fabae *taken from cultures of different ages, when inoculated to leaves abraded with 'Celite'* (*transformed data in bold*)

| | Age of culture (days) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 11 | 19 | 33 | 48 | 107 | (±0·033) |
| Expt. I (±0·057) | | | | | | |
| 'Celite' | 6 | 219 | 184 | 27 | — | — | |
| | — | **2·38** | **2·31** | **1·65** | | | **2·11** |
| No 'Celite' | — | 49 | 8 | 0 | — | — | |
| | | **1·83** | **1·38** | **1·03** | | | **1·41** |
| Expt. II (±0·056) | | | | | | | Mean |
| 'Celite' | 184 | 184 | — | — | 62 | 2 | (±0·028) |
| | **2·31** | **2·31** | | | **1·92** | **1·20** | **1·94** |
| No 'Celite' | 83 | 82 | — | — | 4 | 2 | |
| | **2·01** | **2·01** | | | **1·28** | **1·19** | **1·62** |

## Discussion

Singh (see Hawker, 1950) found that conidia of *Botrytis cinerea* from cultures 16 days old were more active in water, as measured by the percentage germinating and by the growth of germ tubes, than were conidia from younger and older cultures. The *Botrytis* isolate used in the present experiments differs from those used by Singh and by Gilles (1959), respectively, in having no peak of activity. Germination was maintained at a constantly high level until conidia were 30 days old and then progressively decreased, being halved when they were 45 days old; at this stage the *Botrytis cinerea* conidia tested by Brown (1922) would only germinate in

nutrient solutions. Infectivity of *B. fabae* rapidly decreased with age and was halved when conidia were 9 days old (i.e. when cultures were 16 days old).

The relative infectivities plotted in Fig. 2 are calculated assuming that conidia from cultures up to 10 days old all infect. This is, however, untrue because more lesions developed on abraded than on unabraded leaves. Nevertheless, the comparative values remain the same.

Blackman & Welsford (1916) showed that germinating conidia of *B. cinerea* penetrate broad bean leaves by mechanical pressure, and that enzymes breaking down pectic substances (Wood, 1955) become involved only in the post penetration stages of infection. In my experiments it is not clear whether the loss of infectivity, as measured by numbers of lesions, is attributable to the mechanical inability of germinating conidia to penetrate or to the inability to spread subsequently and disrupt the host. Even though glucose, mannose and fructose, but not galactose, favour the production of pectinase by *Pythium de Baryanum* (Gupta, 1956), it would be surprising if *Botrytis* conidia of different ages that are equally able to penetrate could not also equally use host material for subsequent growth and to produce disruptive enzymes. That the loss of infectivity with increasing age is at least partially attributable to the inability to penetrate is suggested by the results of experiments with 'Celite' (Table 4). Conidia from cultures 11 and 19 days old gave forty-nine and eight lesions on intact leaves respectively, but when leaves were abraded so that penetration was easy, approximately equal numbers of lesions (*ca.* 200) developed.

The substances which partially restored infectivity are those that act as good carbon sources for the vegetative growth of most fungi, viz. sucrose, glucose, mannose and maltose. During periods of high humidity, which favour infection by *Botrytis*, nutrients may exude from the leaf into the infection drop by passive exosmosis, increasing the vigour of germination and decreasing the time for infection (Brown, 1922). In addition, the phyllosphere may be affected by plant and insect debris. Broad beans heavily infested with *Aphis fabae* Scop. are often severely attacked by *Botrytis fabae*, the cause of chocolate spot. Honeydew was collected and added to conidial suspensions from cultures *ca.* 25 days old. The numbers of lesions/$\frac{1}{2}$ leaflet increased in two experiments from 6 to 26 and from 3 to 27, respectively—an effect probably attributable to the high concentrations of sucrose and glucose usually found in honeydew (Gray & Fraenkel, 1954; Mittler, 1958).

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# GLOEOSPORIUM FRUIT ROT OF BANANA

By HUSSEIN ELAROSI

*Agricultural Botany Department, Alexandria University*

(With Plate 28 and 5 Text-figures)

*Gloeosporium musarum* was isolated from ripe-fruit rot of banana for the first time in Egypt. Fungal growth and sporulation are described on plain, malt, Czapek-Dox and potato-dextrose agars. The fungus produced black sclerotia in culture as a result of glass-contact stimulus. Appressorium-like cells were formed in terminal and intercalary positions from sclerotial hyphae.

Development of the disease on mature wounded and unwounded banana fruits of the variety *Musa cavendishii* is described. In wound-inoculated fruit aggregated hyphae advanced intercellularly in zigzag lines from the site of inoculation towards the inside of the fruit. From these lines, inter- as well as intracellular invasion developed.

The importance of post-harvest diseases has attracted, in the last few years, the attention of plant pathologists in Egypt. In bananas, cigar-end and Botryodiplodia fruit rots have been recorded in Egypt (El-Helaly, Ibrahim & Elarosi, 1954, 1955).

Gloeosporium fruit rot of banana is known by different names: ripe-fruit rot, anthracnose and black rot (Wardlaw, 1930), and has been studied by Dastur (1916), Simmonds (1928), Wardlaw (1930), and by Simmonds & Mitchell (1940). It is caused by the fungus *Gloeosporium musarum* Cooke & Massee, which apparently cannot enter uninjured green or partly matured fruits in the field, even of the most susceptible varieties; yet completely mature bananas, when placed under proper conditions, became diseased whether injured or not (Wardlaw, 1930). It was proved that fruits can be infected long before becoming ripe and before the disease becomes outwardly visible, but that the fungus remains dormant in the peel till suitable conditions arise for further development (Dastur, 1916; Simmonds & Mitchell, 1940; Chakravarty, 1957).

## Materials and methods

The parasite was first isolated from a banana fruit of the Baladi variety of *Musa cavendishii* diseased with ripe-fruit rot, and then re-isolated using a single-spore technique. Inoculation experiments were carried out on mature fruits of the Baladi variety, using two methods: a spraying method whereby sound fruits were sprayed with a fungal spore suspension, and a wound-inoculation method whereby a disk 3·5 mm. diam. from a 10-day culture of the parasite was placed in a bore 2–3 mm. deep, made by a cork-borer half-way along the fruit length. After inoculation, the bore was plugged with the removed piece of peel. Inoculated and uninoculated fruits were kept in closed humid chambers and incubated at room temperature (15·5–20·3° C.).

Infected fruit tissues were fixed, 13 days after inoculation, in the FAA mixture: 50 ml. ethyl alcohol (95%), 5 ml. glacial acetic acid, 10 ml. formaldehyde (40%) and 35 ml. water (Sass, 1958). Fixed tissues were embedded in wax, and then sectioned and stained with thionin and orange G (Stoughton, 1930).

## Results

### *Isolation of the pathogen*

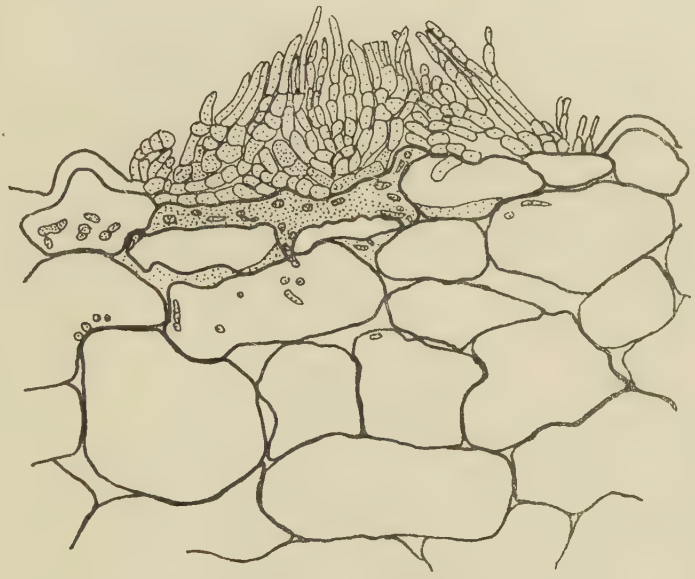
Gloeosporium fruit rot was found around Alexandria in 1957, causing slight losses in the plantation and extensive ones in the market. Overripe fruits were severely infected, especially under high relative humidities. Isolation from diseased fruits revealed the presence of *Gloeosporium*

Text-fig. 1. Teased piece of sclerotium of *Gloeosporium musarum*, showing thick short-celled hyphae and appressorium-like cells. × 800.

*musarum* Cooke & Massee. Fungal growth on plain agar was hyaline with scant sporulation. Spores accumulated in minute heads on scattered short conidiophores. Growth on both Czapek-Dox and malt agars was ivory in colour, and pale pink on potato-dextrose agar. Sporulation on Czapek-Dox, malt and potato-dextrose agars was abundant, in the form of acervuli. Conidia were oblong to ovate and unicellular, but often became septate on germination. Their dimensions were 10·4–23·4 by 3·2–5·9 $\mu$, with a mean of 14·6 by 4·6 $\mu$. Spore dimensions agree with those measured by Toro (1922), which were 10·0–24·0 by 3·0–6·0 $\mu$, with a mode of 11·0–17·0 by

3·5–4·5 $\mu$, and differ in width from those measured by Ashby (1931), which were 10·0–20·0 by 5·0–7·0 $\mu$ (Wardlaw, 1935). Black sclerotia were formed on both the lower surface and the periphery of the agar, in contact with the glass. Thick-walled terminal and intercalary dark-brown, appressorium-like cells are formed from the sclerotial hyphae (Text-fig. 1; Pl. 28, fig. 3).

Some workers have recorded strains of *G. musarum* forming black hemispherical or irregular stromatoid outgrowths of the surface mycelium, with a pseudoparenchymatous structure (Wardlaw, 1935). The black sclerotia observed here differ from the black stromatoid outgrowths in their lack of conidiophores, and also in the formation of appressorium-like cells.



Text-fig. 2. Acervulus of *Gloeosporium musarum* formed on the surface of infected banana fruit. ×467.

*Development of the disease*

Early symptoms of the disease, when mature fruits were inoculated by spraying, appeared on the fifth day after inoculation, as small, dark-brown to black, circular spots. These spots enlarged rapidly, and coalesced to form large, slightly depressed areas, dark-brown to almost black in colour. Later on, i.e. 15–20 days after inoculation, the fruit pulp became softened and the diseased fruits were covered with minute pink lesions formed by the acervuli and with the wet spore masses, which gave the diseased fruits a slimy appearance. Dimensions of conidia formed on fruits did not differ much from those of conidia formed on artificial media. They were 10·4–14·3 by 3·2–5·2 $\mu$, with a mean of 12·5 by 4·4 $\mu$.

Early symptoms of the disease, when fruits were wound-inoculated,

appeared in the first few days, as a brown zone round the site of inoculation surrounded by a yellow one. After 9 days the rotted tissue became brown, grading gradually to red and then to yellow. After 12 days the pink-coloured acervuli appeared round the site of inoculation and the gradation of colour disappeared; the brown-coloured rotted skin was followed directly by the yellow colour.

Text-fig. 3. Cross-section of infected banana fruit, showing the destruction of tissue near the wound and the accumulation of hyphae in a zigzag line. ×41.

### *Histological studies*

Sections of infected fruits inoculated by the spraying method showed that epicarp, hypoderm and outer mesophyll were invaded by the fungus. Inter- as well as intracellular invasion was observed. Radial cell-walls in

the outer layers were compressed, and acquired a corrugated appearance. Fibre bundles were longitudinally split in many parts. The outer parts of split fibre bundles with all outer layers were sometimes torn out. Oleoresin cells, especially those near the fibre bundles, and the fibro-vascular ones were expanded. Oleoresin cells, cells containing raphides, and fibre cells were normally free from the parasite.

Acervuli were formed on different parts of the epicarp of heavily invaded fruits, and were borne on flat, shallow stromata of pseudoparenchyma formed below the epicarp, causing the rupture of this layer at different points. From the stroma a compact mass of parallel septate conidiophores, bearing single unicellular condia, was produced (Text-fig. 2).

Text-fig. 4. A part of the zigzag line formed by intercellular hyphae of *Gloeosporium musarum*, and showing some intracellular hyphae. × 360.

In fruit infected by the wound-inoculation method, described above, the fungus accumulated at the wound, attacked heavily all the tissues damaged by the cork-borer, and then invaded the sound tissues. Transverse sections through the site of inoculation showed one or more zigzag lines of accumulated hyphae in the intercellular spaces, starting from the damaged tissue towards the endocarp. These intercellular hyphae sent solitary intra- and intercellular hyphae into the surrounding tissues (Text-figs. 3, 4; Pl. 28, figs. 1, 2).

When two zigzag lines of accumulated hyphae occurred near each other, the tissues between them were less attacked by the parasite than the tissues on either side. This phenomenon may be due to iso-antagonism of the pathogen (Waksman, 1937), resulting from the accumulated by-products of the two hyphal lines (Pl. 28, fig. 2).

In the endocarp the fungus attacked vigorously nearly all the cells, causing their destruction. Especially towards the inside of the fruit, cell

walls disintegrated and hyphae accumulated around starch grains (Text-fig. 5). These results confirm the finding of Simmonds & Mitchell (1940) that mycelium of *Gloeosporium musarum* was at first largely intercellular, and later became intra- and intercellular.

Cross-sections cut away from the site of inoculation showed that, while the outer parts of the fruits were nearly free from the fungus, the inner parts were infected.

Text-fig. 5. A section through the endocarp of an infected banana fruit, showing disintegration of cell walls and accumulation of hyphae round starch grains. × 1250.

## Discussion

Gloeosporium fruit rot is a world-wide disease, but no previous record of the disease in Egypt has been found. Most of the work on the histology of infection has been concerned with latent infection and with penetration (Simmonds & Mitchell, 1940; Chakravarty, 1957).

In the present work, a peculiar mode of invasion was observed, following upon wound inoculation, in which the fungal hyphae advanced collectively to the inside. The mechanical power produced by such an aggregate of hyphae is presumably greater than that produced by a solitary hypha. Mechanical invasion of a nearly similar type was observed by the author in potato tubers infected with *Rhizoctonia solani*. Hyphae of *R. solani* accumulated in a certain cell, exerting pressure on the cell wall and causing its rupture (Elarosi, 1957).

Such an invasion by an accumulation of hyphae in wounded fruits and not in the sound ones may be due to retardation of penetration in the latter. Penetration of sound tissues proceeds when ripening sets in, rendering the tissues more susceptible.

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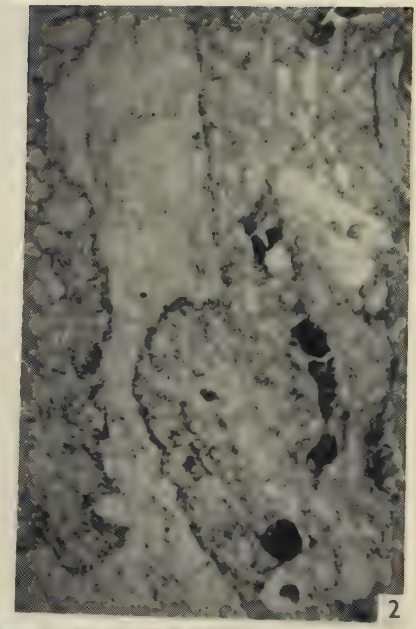

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## EXPLANATION OF PLATE 28

Fig. 1. Cross-section of banana fruit infected with *Gloeosporium musarum*, showing destruction of tissue near the wound and the accumulation of hyphae in a zigzag line (× 76).

Fig. 2. Section of infected banana fruit, showing invasion by *G. musarum* in two zigzag lines (× 76).

Fig. 3. Teased piece of sclerotium of *G. musarum*, showing short-celled hyphae and appressorium-like cells (× 795).

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*Trans. Brit. mycol. Soc.* **43** (4), 688 (1960).

# A CORRECTION

In my previous note (*Trans. Brit. mycol. Soc.*, 1952), in which I detailed a cotton blue-lactophenol method used by Dr Marcelle Le Gal for showing spore markings in Operculate Discomycetes, I stated that her technique called for boiling to dissolve the dye, but that I was 'not convinced that boiling the lactic acid to dissolve the dye is advisable, as there is a tendency for small precipitates to form on standing'. In conversation with me at the International Botanical Congress in Paris during 1954, Mme Le Gal advised me that I had translated the words 'en broyant' into 'boiling', instead of into 'grinding (or crushing)'. This error is inexcusable. My supposed improvement—not boiling—is thus not an innovation but part of her original technique. A rectification should have been made earlier—even though anyone following either Mme Le Gal's French text or my so-called improvement would have had identical results—since she has recently indicated that my faulty translation 'nous impute une erreur que nous n'avons pas commise', (*Bull. Soc. Mycol. Fr.* **74**, 170, 1958). The error, let it be made clear, is wholly Korf's, not hers.

The cotton-blue technique, or Korf's permanent mount modification (cotton blue in water) also brings out spore markings in Phycomycetes, e.g. in the zygospores of *Conidiobolus ?brefeldianus* Couch, which Dr M. A. Rosinski and I isolated in Highlands, North Carolina, from apothecia of *Peziza proteana* (Boud.) Seav. f. *proteana*. One of the inner walls is shown to be provided with prominent wart-like markings which stain dark blue.

One of my former students, Mr A. R. Christiansen, demonstrated blueing of the markings in uredospores of *Puccinia graminis* Pers. ex Pers.; in basidiospores of *Astraeus* sp., *Ganoderma lucidum* (Leyss. ex Fr.) Karst., and *Tulostoma* sp.; in ascospores of *Corynelia* sp.; and in zygospores of *Conidiobolus brefeldianus*. Blueing of the spore markings is also common in the Hypocreales, in the Tuberales, and (rarely) in the Helotiales.

RICHARD P. KORF

*Department of Plant Pathology*
*Cornell University*
*Ithaca, N.Y.*

*Trans. Brit. mycol. Soc.* **43** (4), 689–695 (1960).

# BRITISH RECORDS

43. *Trichophaea abundans* (Karst.) Boud. Plentifully on charred wood at a bonfire site near Congleton, Ches., Nov. 1943. Herb. W.D.G. 269 and Herb. Kew. Well characterized by its small size (to 3 mm.), short hairs (to 160 $\mu$) and the two relatively large polar guttules in the small spores 13–16 × 7 $\mu$ (Fig. 1). In Herb. Kew. there is a collection on bits of charred wood (H. C. Hawley, Oban, Sept. 1908) under the name *T. lecothecioides* which Dr Dennis has allowed me to examine (Fig. 2). This seems to me to be another collection of *T. abundans*. The small apothecia are well preserved; they have a whitish disk fringed with short brown hairs and in no way resemble the dry appearance of *T. lecothecioides* as given by Rehm (*Rabenh. Krypt. Fl.* III, p. 1043: 'trocken einem Pyrenomyceten gleichende Art, fast geschlossen und braun schwarz, deren Behaarung erst unter dem Mikroskop deutlich erkennbar ist').

44. **Trichophaea hemisphaerioides** (Mont.) comb.nov. (*Lachnea* h. Mont., in *Bull. Soc. roy. Bot. Belg.* **36**, 21 (1897)). Good material from: Borrowdale, June 1956 (D. A. Reid, Herb. Kew.); Malham Tarn, Yorks, Sept. 1958 (Herb. W.D.G. 1278); Chatsworth, Derby., Oct. 1958 (Herb. W.D.G. 1322). A much larger species than *T. abundans* and growing on the soil of old, moss-invaded bonfire sites; apothecia 6–10 mm. and sometimes reaching 17 mm. The hairs reach 450 $\mu$ and are multi-septate (to 15); the spores have a pair of small polar guttules—very constant in all collections seen and noticeably smaller than those of *T. abundans* (Fig. 3).

45. *Trichophaea woolhopeia* (Cke & Phill.) Boud. Good material from: Longshaw, Derby., Sept. 1956 (Herb. W.D.G. 1120); Reading, Berks, Sept. 1958 (Herb. W.D.G. 1317). A small species (to 5 mm.) of bonfire sites and charred debris. The large spores 20–23 × 14–15 $\mu$ have a large guttule and sometimes several small ones; the hairs reach 260 $\mu$ and are rarely more than 3-septate with the septa usually in the basal part (Fig. 4).

46. *Trichophaea gregaria* (Rehm) Boud. Covering several sq.in. of a disintegrated coniferous log near Coleford, Forest of Dean, Aug. 1955 (Herb. W.D.G. 1940 and Herb. Kew.). A small species (to 5 mm.) of soil and much decayed plant debris. Spores subfusiform 24–28 × 11–12 $\mu$ with a large, generally ellipsoidal central guttule and a mass of smaller ones at each pole; the spore surface shows numerous minute dark dots in lactic blue but these do not roughen the spore profile (Fig. 5).

47. *Trichophaea pseudogregaria* (Ricken) Boud. Widely spread on bare soil under dense *Mercurialis perennis* cover at Malham Tarn, Yorks, Sept. 1958 (Herb, W.D.G. 1279). Spores 22–24 × 10–11 $\mu$ with one or more rather large guttules and many smaller ones; spore surface with many

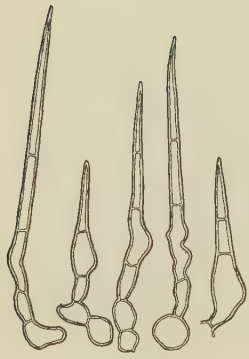

Fig. 1

Fig. 2

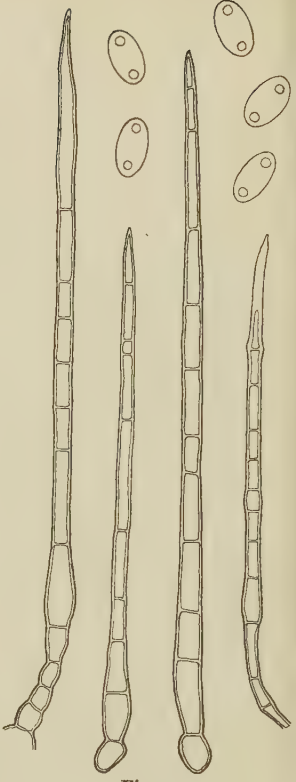

Fig. 3

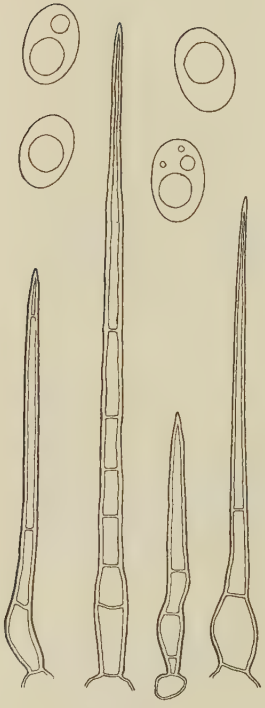

Fig. 4

Fig. 5

Fig. 6

Figs. 1–6. 1, *Trichophaea abundans:* spores and hairs; 2, '*T. lecothecioides*': spores, hairs, ascus and paraphyses; 3, *T. hemisphaerioides:* spores and hairs; 4, *T. woolhopeia:* spores and hairs; 5, *T. gregaria:* spores and hairs; 6, *T. pseudogregaria:* spores and hairs.

conspicuous warts which project about 1 $\mu$ from the spore profile (Fig. 6).

48. *Aleuria apiculata* (Cooke) Boud. Two good specimens on a log lying in the water of Malham Tarn, Yorks, Sept. 1958 (Herb. W.D.G. 1299). Apothecia cupulate, 10 mm. across; disk dark olivaceous; externally purplish brown. Spores 22–27 × 9–10·5 $\mu$ (without apiculus), with an apiculus, sometimes forked, about 2 $\mu$ long at each end; epispore finely warted, the warts giving a spinose appearance to the spore profile; mature spores golden-brown. Upper part of the ascus wall and the operculum strongly blued by Melzer reagent (distinction from *Discina*—also with apiculate spores).

49. *Aleuria granulosa* sensu Boudier, *Icones mycol.* Pl. 272. Scattered about the soil of a bonfire site in Chatsworth Pk., Derby., Sept. 1958 (Herb. W.D.G. 1319). To 12 mm. across, flat with a yellow-brown disk and upturned finely crenulate margin. Spores 21–22 × 11–12 $\mu$; epispore smooth. Paraphyses with a head up to 9 $\mu$ across, greenish and filled with blackish granules when seen in lactic blue.

W. D. GRADDON, '*Beechwood*', *Congleton, Cheshire*

50. **Leccinum percandidum** (Vassilk.) comb.nov. (Syn. *Boletus percandidus* Vassilk., *Sovetsk Bot.* no. 2 (1944), p. 27; *B. versipellis* var. *percandidus* Vassilk. 1948, Moscow; *Krombholzia rufescens* var. *percandida* Pilát, 1951; *K. aurantiaca* f. *percandida* (Vassilk.) Vassilk., *Not. Syst. Sect. Cryptog. Inst. Bot. Acad. Sci. U.R.S.S.* **11** (1956), p. 139, fig. 5.)

*Cap* 75–120 mm., hemispherical then convex, almost white with a distinct rosy tint, then distinctly rose, finally reddish brown, resembling the colour of *Collybia maculata*; minutely fibrillose tomentose, splitting up into very minute scales on expanding. Margin irregular, the cuticle of the pileus overhanging the tubes as an irregular 'veil'. Cuticle bruises purple brown immediately on handling. *Stem* 150–160 × 35–40 mm., firm, pure white, reddish brown when bruised about the middle and lower part but blue green at the base, often strongly so and especially when eaten by slugs or when young; covered with large white floccose scales to the base, those at the apex smaller, distributed on rounded ribs, slightly rugulose, the scales darkening to pale or darker brownish in mature specimens when bruised. Mycelium at stem base white, immediately bruising blue green. *Tubes* adnate, shorter nearer the stem, white in young specimens, then faintly ochraceous due to maturing spores, slightly lilaceous when cut or bruised, 10–15 mm. long when mature. *Pores* small, equal, round, never greater than 0·8 mm. diam., white then with ochraceous tint on maturing, distinctly lilaceous when bruised. *Flesh* white, immediately rose-lilaceous when cut, then becoming more purplish, finally purple black in stem apex but tinted yellow brown in places, blue-green near cuticle of stem, firm, 2–3 cm. thick at centre of cap, compact. *Smell* pleasant and *taste* mild, not distinctive. *Spores* snuff brown in mass, subfusoid, slightly hooked at one end (11)12–14 × 4–5 $\mu$. *Basidia* 4-spored. *Cystidia* subcylindric or clavate-

elongate, smooth. *Cap cuticle* with filamentous cells with clavate or fusiform ends.

In *Pinus-Betula* stand. Under *Betula*, Badger Falls, Tomich, Strathglass, Inverness-shire. Coll. P. D. Orton and R. Watling, 30 Aug. 1957.

This fungus appears to be close to *L. aurantiacum* (Bull.) S. F. Gray but differs in the white or very rose tinted pileus, the blue-green colour change in the stem cuticle and the different colour change sequence in the main body of the flesh. In these characters it resembles *L. testaceoscabrum* (Secr.) Singer. Vassilkov *in litt.* states that from the distinction of the interesting rose tint of this collection one could possibly consider it an independent, though related form. Because the specimens agree with Vassilkov's fungus so closely, except for this rose tint, it has been decided to adopt this name. It is proposed, however, to transfer it to *Leccinum* S. F. Gray because of its affinities to *L. aurantiacum* and *L. testaceoscabrum.*

51. **Leccinum holopus** (Rostk.) comb.nov. (Syn. *Boletus holopus* Rostk. apud Sturm *Deutschland's Flora*, 1828; *B. scaber* var. *niveus* Fr. Opat.)

*Cap* 40–70(100) mm., hemispherical, then expanding, almost pure white, dirty white, chamois, very pale buff or pale pallid, but nearly always with a glaucous green tinge in mature specimens and often in young ones; the tint often increasing in intensity, occasionally making the cap quite yellow green; minutely tomentose at first, soon becoming smooth, but often slightly viscid when moist; margin of cap not or scarcely overhanging tubes in a regular 'veil'. *Stem* 80–110(120) × 8–15(15–30 base) mm., white or only faintly dirty, becoming slightly greyish with age; covered with large white floccose scales distributed on rugulose lines; white tomentum at the slightly swollen base obviously connected with the habit of growing in damp places; scales at stem apex discoloured slightly cinnamon with age by the mature spores; mycelium at base of stem white, immediately turning blue green on bruising. *Tubes* adnate with occasionally small tooth, white, dirty white or pallid unchangeable 9–15 mm. long. *Pores* small white or pallid, not more than 1 mm. diam., round, bruising possibly slightly yellow cinnamon when mature. *Flesh* with mild taste and pleasant odour, not distinctive, white, changing to blue-green at stem base, often slightly pinkish elsewhere or unchangeable; no blue-green in cap flesh and rarely high up the stem. *Spores* snuff-brown-cinnamon in mass, subfusoid, slightly hooked 17·5–20(21) × 5·5–7 μ. *Basidia* tetraspored. *Cystidia* smooth, subcylindrical. *Cap cuticle* filamentous with cylindrical cells.

Single, rarely more than one in close vicinity; all collections amongst sphagnum, usually sphagnum bogs under or near *Betula*. Badger Falls, Tomich, Strathglass, Inverness-shire, 27–30 Aug., 1957 coll. R. Watling; Plodda Falls, Tomich, 29 Aug. 1957, coll. P. D. Orton and R. Watling; woods opposite Plodda Falls, 2 Sept. 1957 coll. R. Watling; Pollanbuidhe, Glen Affrich, 3 Sept. 1957, 4 specimens coll. P. D. Orton and R. Watling; wood near Cannick, Strathglass, 5 Sept. 1957, coll. R. Watling; Badger Falls, Tomich, 6 Sept. 1957, 8 specimens growing under same conditions as *Suillus flavidus*, coll. P. D. Orton and R. Watling; Loch Craiglush nr. Dunkeld, Perthshire, 18 Sept. 1957, coll. P. D. Orton.

This species is sufficiently distinct. Its pale colours, colour changes of the flesh when mature, colour of the scales on the stem and the habitat make it easily separable from *L. scabrum*, even in the field.

It appears to be the counterpart of Vassilkov's species described above, *L. holopus* being related to *L. scaber* as *L. percandidum* is to *L. aurantiacum*, the faint tint in both, glaucous green and rose pink respectively, is often absent and the sporophores appear purely white. Though Fries's var. *niveus* of *B. scaber* was published earlier it was not raised to specific rank until after Rostkovius had described the same species under *holopus* thus giving this epithet priority.

I would like to thank Prof. Vassilkov for sending information on members of the Boletaceae, especially *B. percandidus*. Material of both species has been deposited in Herb. Kew, and also in Wat. Herb. under the accession numbers 193C *L. percandidum* and 194C *L. holopus*.

52. *Lepiota gueguenii* Sacc. sensu Locq.

*Cap* 18–20 mm. diam. with possibly a small umbo and with a sulcate striate margin up to $\frac{3}{4}$ way to the disk, very pale lemon yellow or starting ivory white flushed yellow, then pale lemon yellow, finally brighter with flush of yellow ochre. Mealy in appearance from being covered in similarly coloured farinous granules which come off on to anything which touches the cap. Possibly more greeny sulphur yellow with age, flushed slightly yellow ochre when drying. Umbo and disk more yellow than margin. Meal made up of globose cells 20–30 $\mu$ diam., smooth. *Stem* 30–40 × 3–4 mm., with small pale yellow collar-like membranous, mealy ring directed upwards on the upper part. Stem mealy, especially below the ring, minutely fibrose-striate at apex and towards the base. *Flesh* white, faintly pale lemon. Taste and odour not distinctive, pleasant. *Gills* ivory white, faint tint of sulphur yellow, becoming stronger yellow, even becoming flushed faintly chrome yellow in older specimens. Free, not forked or interveined, crowded. *Spore mass* white. *Spores* 4–5 × 5·5–6·5 $\mu$ smooth, without germ pore, pseudoamyloid. *Cystidia* both facial and marginal absent. *Basidia* 4-spored. *Mycelium* pale sulphur yellow.

Growing on peat compost introduced into greenhouse, The University, Sheffield, 8 July 1959, Wat. Herb. 198C. Persistent under these conditions for a number of days.

A distinct, fairly fragile agaric with a delicate appearance, recognizable by the pale lemon yellow colour, the meal on the cap and lower part of the stem made up of globose cells. Obviously an alien though worthy of record.

53. *Mycena longiseta* Höhn.

*Cap* 3–5·5 mm., conical, convex on expanding, $\frac{3}{4}$ striate, pale grey with faint brownish tinge, especially at the disk. Mat or slightly shining at edge, margin more whitish. Typically covered with aseptate, thick-walled, pointed hairs. Drying more whitish grey, with hairs up to 150 $\mu$ in length, rarely greater, but quite variable. Hairs slightly swollen towards the base either straight or horn shaped. Cap cuticle elastic, separable. *Stem* 15–27 × 0·5–0·7 mm., very pale grey almost white translucent, scattered

with a few hairs of the same structure as those of the cap; these increase in number towards the base which is abruptly swollen to form a small but distinct whitish basal disk 3–5 mm. diam.; straight or curved equal or possibly slightly tapering towards the apex. *Gills* free, greyish white. *Spore mass* shite. *Spores* smooth, pseudoamyloid, ellipsoid with apicules 6–8 × 3–4 μ. *Basidia* 4-spored, *Cheilocystidia* small, smooth, slightly cylindrical.

Growing on old alder stump (*Alnus glutinosa*) amongst moss, Hardcastle Craggs, Hebden Bridge, Yorks, 20 Oct. 1956, Wat. Herb. 19C. Collected independently by W. D. Hincks and R. Watling, at subsequent intervals by R. W. Under *Calluna*, on conifer debris (*Pinus sylvestris*), Rothiemurchus Inverness-shire 10 Sept. 1957, collected independently by D. Henderson and R. Watling. This species is easily recognizable by its small size, prominent setae covering the cap and hairy basal disk.

54. *Coprinus miser* (Karst.) Karst.

*Cap* 1–3 mm., high, ovoid, 1–2 mm. across when mature, delicate, ruddy brown with a distinct brick red or orange cast, when first appearing; soon discolours and finally becomes pale sepia or vivid grey flushed brown; striate from margin almost to the very disk, rarely expanding but remaining almost paraboloid. *Cap cuticle* made up of ovoid-globose cells 20 μ in width. *Stem* short, then expanding whitish or slightly grey, apex concealed, 0·5 mm. diam. and < 15 mm. long, glabrous. *Gills* compact and crowded, pale grey tinged later lilaceous, then purple. *Spore mass* black-purple. *Spores* black, smooth, somewhat flattened in one direction 7–9 × 7–8 × 5–6 μ.

Collected Wathe Wood, Pateley Bridge, Yorks. On cow dung in field, abundant, 12 Apr. 1959, Wat. Herb. 195*c*. A delicate and pygmean *Coprinus*, easily recognizable by the cap being bright orange ruddy brown at first and distinctive spores.

I should like to thank P. D. Orton for his continual help, encouragement and advice and also F. B. Hora, D. Henderson and W. D. Hincks must be thanked for their notes on the collections of *Mycena longiseta*.

R. WATLING, *The University, Sheffield*
(now at *Royal Botanic Garden, Edinburgh*)

55. *Ascodesmis porcina* Seaver (*Mycologia*, **8**, 3, 1916) appeared on unidentified mammal's dung, collected at Bury Hill, Sussex, 27 Apr. 1960 (Herb. I.M.I. 80559), after about 2 weeks incubation in a damp chamber.

Asci, 54–60 μ × 20–27 μ, were smaller than indicated in the original description; ascospores were subglobose, 10–12 × 8–10 μ, brown and ornamented with warts, as described by Seaver. Ascospore ridges were not well defined except along the surface of the germ slit.

56. *Ryparobius polysporus* (Karst.) Sacc. (*Fungi Fenn.* 665, 1867; *Syll. Fung.* **8**, 539, 1889) was isolated from (*a*) rabbit dung, Richmond Park, Surrey, 1956 (Herb. I.M.I. 67944); (*b*) hare dung, Royal Lytham and

St Anne's Golf Course, Lancs, 1958 (Herb. I.M.I. 75299). The light brown apothecia were barely visible with × 10 magnification and appeared on streak plates made from the above material. They were never observed on the incubated dung and, for this reason, the species has been probably overlooked in the past and not previously recorded in this country.

Seaver (*The North American Cup Fungi* (*Operculates*), p. 146, 1928) reports few asci to each apothecium, but I.M.I. 67944 and I.M.I. 75299 produced as many as 16 and 12 asci per apothecium, respectively.

JEAN GUNNELL, *Commonwealth Mycological Institute, Kew*

57. *Uromyces minor* Schroet. On *Trifolium molinerii* Balb. Collected 9 Apr. 1959, Isle au Guerdain, Portelet Bay, Jersey.

This clover occurs in the Channel Islands only in Jersey at two isolated localities where but a few hundred plants exist. The bright ochre-yellow, irregular sori on the leaves and petioles contained abundant aecidiospores, which were followed by occasional teleutospores; uredospores could not be found in any of the several specimens collected. *T. molinerii* is close to *T. incarnatum*, being considered by some as the wild material from which the latter was selected for agricultural use. R. González Fragoso, in *Flora Iberica*, *Uredinales*, II, p. 89 (1925), reported *U. minor* on *T. incarnatum* and *T. montanum* in eastern and northern Spain. The material from Jersey has been examined by Dr R. W. G. Dennis and Dr I. Jørstad, who say that in view of the absence of uredospores and the nature of the host they see no reason why the rust should not, at least provisionally, be named *U. minor*.

Aecidiospores (10 measured) were 17–22 × 12–19, av. 19 × 16 $\mu$, and teleutospores (40) 22–28 × 13–22, av. 24 × 18 $\mu$, l/b 1·3, dimensions which are slightly larger than those given by Guyot (*Encycl. mycol.* XXIX, *Les Uredinées*, III, *Uromyces*, pp. 339–348, 1957), viz. 14–20 × 10–20 $\mu$ and 16–24(15–28) × 13–18(10–20) $\mu$, l/b 1·1–1·3. Guyot stated that in Europe aecidiospores are produced in mid-May and teleutospores in June but in America both occur at the same time and earlier on the same leaves. He also reported *U. minor* to be absent from N.W. Europe and the British Isles but listed 10 *Trifolium* spp. (but not *T. molinerii*) recorded as hosts in Europe and 33 spp. in western U.S.A. Material has been deposited in Herb. Kew and Herb. I.M.I.

L. C. FROST, *Department of Botany, University of Bristol*

# PROCEEDINGS, 1960

## MEETINGS

9 January 1960. Meeting held at the London School of Hygiene and Tropical Medicine, London, W.C.1, at 11.0 a.m. with the President, Mr E. C. Large, in the Chair. This meeting, arranged by the Plant Pathology Committee, constituted the second half of a two-day Symposium, held jointly with the Association of Applied Biologists, entitled 'the nature and exploitation of crop plants' resistance to disease'.

### Variation and race specialization in the pathogens

D. Lewis. General introduction.

A. C. Hastie. Variation in *Verticillium albo-atrum* from hop.

A search has been made for mechanisms responsible for variation in this fungus which has no known sexual stage. Several authors have shown that most of the conidia produced by *V. albo-atrum* are uninucleate, and since it was found that cultures of some hop isolates established from single hyphal tips yielded conidia which produced either dark or hyaline colonies it seemed likely that heterokaryosis occurred. This was confirmed by using nutritionally deficient mutants obtained following ultraviolet irradiation, and also a spontaneous mutant resistant to acriflavine. Spores of a hyaline resistant strain, requiring both methionine and biotin, were mixed, with conidia from a black, sensitive strain which was unable to synthesize adenine, and the mixed inoculum incubated on unaugmented Czapek-Dox agar medium. This mixed inoculum produced slow growing sectors, and some of the hyphal tips from these subsequently yielded conidia of both parental types.

Prototrophic conidia were recovered at a low frequency from such a heterokaryon by spreading a dense spore suspension on Czapek-Dox agar. These prototrophs were sensitive to acriflavine, but produced resistant strains, some of which showed other characters included in the original inoculum. All the mutant phenotypes have been recovered indirectly from a single conidium. Because this had a high probability of being uninucleate, it may be inferred that it was heterozygous, and that a system essentially similar to parasexuality can give rise to novel progeny in *V. albo-atrum.*

R. D. Tinline. Pathogenic and cultural variation in *Cochliobolus sativus.*

The fungus *Cochliobolus sativus*, the perfect stage of *Helminthosporium sativum*, is known to be highly variable. Recently, strains were obtained that differed in spore colour, resistance to ultraviolet irradiation, mycelial pigmentation, degree of sexual compatibility, virulence, sporulation and other cultural characters. In the laboratory, three mechanisms appeared operative in extending the range of variation in the species. New strains differing from the parents in spore colour, degree of sporulation and virulence arose through mutation. These changes occurred in mono-ascosporic isolates and they were inherited through the perfect stage. Heterokaryosis with its perpetuation through the multinucleate, multicellular conidia was the most plausible explanation for the recovery of two distinct cultural types from successive single-spore generations. Presumably, the heterokaryon was initiated by mutation in a spore that was taken from a mono-ascosporic culture. Hybridization also may contribute to variation in *Cochliobolus sativus*. Progeny that were isolated from crosses between a white-spored, poorly sporulating, weakly virulent strain and a dark-spored, densely sporulating, highly virulent strain, possessed various combinations of the parental characters. Evidence that new strains for virulence

may arise through sexual recombination was obtained by mating isolates having similar degrees of virulence. Approximately 25 % of the progeny from two such matings had significantly greater or less virulence than the parents, whereas only 3 % of single spore cultures from the parental clones exhibited a significantly altered virulence.

## M. A. Watson. Interaction, or genetic recombination, between potato viruses Y and C.

See *Virology*, **10**, 211–232, 1960.

## D. A. Doling. Physiologic race surveys.

The recognition of physiologic races is concomitant with the accumulation of a range of genetic material of the host plant. Their study forms an essential part of a variety-testing programme, as the occurrence and distribution of physiologic races indicates which sources of resistance are still functional and thus the probable behaviour of a variety in a particular locality. A system of nomenclature with a genetic basis relating races to resistance genes and to one another is of immediate practical value, and provides a fundamental classification of use in further work.

Surveys designed to determine the relative frequency of races are liable to two major deficiencies. First, in addition to the normal sampling errors, others may arise through lack of attention to the biology of host and pathogen. Studies of *Phytophthora infestans* have shown that most races occur where there is the greatest genotypic variation, and different races appear as the season progresses. In some crops varietal resistance may have an important influence on the races present, as in *Puccinia glumarum* in winter wheat. This type of survey does not account for different levels of infection in partially resistant varieties and mere 'numbers of isolates' can give a very distorted picture of the race situation. Secondly, the data so obtained are not the most suitable for the plant breeder. With airborne pathogens the relative frequency of races can quickly change, and the considerable amount of work involved in completing a balanced survey rapidly becomes of little import. The most valuable information is not relative frequency, but the presence or absence of a race in a particular locality.

The pattern now being adopted at the N.I.A.B. is to sow small nursery plots of varieties selected for their susceptibility, differential resistance or complete resistance to *Puccinia glumarum* at many centres throughout the country. By careful and regular observation, records of the first occurrence of the disease on each variety can be made, and the subsequent development of the epidemic followed. Isolates from these plots can be identified on the differential hosts and thus the number of races, their distribution and their contribution to an epidemic each season can be studied.

## C. C. V. Batts. Physiologic specialization in cereal rusts and smuts.

Yellow rust and loose smut were chosen to illustrate aspects of the problems involved in determining race identity and the range of host reactions.

*Yellow rust.* Information on the races present in this country is available from 1946 until the present. Fewer races have been identified in recent years, and biotypes of them (2B and 8B) are now important. The widespread distribution of these biotypes is related to the wheat varieties grown commerically. The choice of differential varieties was discussed, and also seedling and adult plant reactions. The scope of the International Trials in West Europe was indicated.

*Loose smut.* Races were briefly dealt with, and then details of the types of reaction obtained in wheat varieties inoculated with certain races were given. The actual location of the mycelium in the embryo is apparently the deciding factor in the type of reaction produced.

W. Black. Races of *Phytophthora infestans* and resistance problems in potatoes.

The first indications of the mutability of *Phytophthora infestans* occurred in 1932, when blight appeared on hybrid seedlings of *Solanum demissum* that had hitherto been regarded as immune. Since then, with the help of resistance genes from *S. demissum*, a differential host series has been built up, by means of which it is possible to distinguish sixteen different races of the parasite. This host series has been adopted as the basis of the international system for classifying races of the fungus.

The above host series is based on four R genes in their various combinations, and the reaction they control is hypersensitivity. It is now apparent, however, that the species *S. demissum* possesses one, and probably two, additional genes different from those already known. With six such genes it should be possible to distinguish sixty-four different races of the fungus. So far, twenty different races have been recorded, and it is likely that this number will be greatly increased when further gene combinations have been bred and added to the host series.

Race surveys show a close correlation between the distribution of R genotypes and the distribution of the more complex races of the fungus. In the presence of R genotypes the fungus is capable of developing appropriate complex races, but in their absence it tends to revert to simpler forms. The exact mechanism by which these changes take place is not clear. The fungus may be heterokaryotic; it may have a parasexual cycle similar to that observed in *Fusarium*, *Aspergillus*, etc.; or genetic interchange between zoospores may occur.

Since plants with no R gene, or with the same R gene constitution, may differ widely in the amount of damage incurred, it is clear that an additional form of resistance is in operation. This is known as 'field resistance'. It is inherited in polygenic fashion and appears to be effective against all races.

The blight population in Mexico is known to comprise two intercompatible groups of races, a situation which has not been found elsewhere. This population is so diverse that, although some potato species exhibit a very high degree of field resistance, none has remained entirely free from damage.

In view of the wide variability of the pathogen, research is now directed more towards the utilization of the high levels of field resistance available in wild species. The combination of such resistance with the R genes should provide immunity from some races and a valuable degree of resistance to all.

## SPECIAL GENERAL MEETING

12 March 1960. A Special General Meeting was held in the Botany Department, Birkbeck College, London, W.C. 1, at 2.0 p.m. The President, Mr E. C. Large, in the Chair. On the proposition of Council, the proposed new Rules of the Society, which had been approved at the 1959 Annual General Meeting, were ratified *nem. con.*

12 March 1960. Conversazione held in the Botany Department, Birkbeck College, London, W.C. 1, at 2.15 p.m. The President, Mr E. C. Large, in the Chair. The following demonstrations were displayed:

1. G. M. Butler (Birmingham). 'Cellophane' and mycelial growth in *Psathyrella disseminata.*
2. E. W. Buxton (Rothamsted), D. A. Doling (N.I.A.B., Cambridge). *Fusarium* wilt of peas.
3. E. W. Buxton, A. C. Hastie, R. D. Tinline (Rothamsted). Genetic variation in plant pathogenic fungi.
4. R. C. Codner, B. C. Platt (Antibiotics Research Station). The light-induced production of carotenoid pigments by *Cephalosporia.*
5. R. C. Codner (Antibiotics Research Station). Ascocarps of *Emericellopsis terricola* var. *glabra* Van Beyma (Eurotiales).

6. R. Cooke (Birmingham). The agar disk method for the direct observation of the behaviour of indigenous populations of nematode-trapping fungi in the soil.
7. J. Daniels (Cambridge). *Chaetomium piluliferum* sp.nov., the perfect stage of *Botryotrichum piluliferum* Sacc. & March.
8. D. A. L. Davies (Salisbury). The preservation of macrofungi in the dry state.
9. M. P. English, G. Jeffree (Royal Hospital, Bristol). The dissemination of tinea pedis.
10. E. H. Evans (Belfast). Observations on a number of isolates of *Mucor ramannianus* Möller.
11. R. N. de Fonseka (Birkbeck). Some pure cultures of chytrids.
12. M. E. Bunce (Rothamsted). A thermophilic fungus from mouldy hay.
13. E. Griffiths, D. Jones (Aberystwyth). Occurrence of cellulose-decomposing fungi in the soil.
14. E. Grossbard (Grassland Research Institute). Tracer techniques in mycology with special reference to autoradiography.
15. G. Hadley (Glasgow). The light-induced sporulation of *Trichoderma*.
16. E. D. Hamilton (Wright-Fleming Institute). Unidentified flying objects.
17. J. E. Harper, J. Webster (Sheffield). Some coprophilous pyrenomycetes.
18. C. T. Ingold, S. A. Hadland (Birkbeck College). Analysis of ascus-jets.
19. W. R. Jarvis (Scottish Horticultural Research Institute). An apparatus for the study of hygroscopic responses in fungal conidiophores.
20. W. R. Jarvis (Scottish Horticultural Research Institute). The structure of the conidiophore of *Botrytis cinerea* Pers.
21. E. C. Large (Harpenden). Some water-colour sketches of agarics.
22. J. Lythgoe (Cambridge). Morphogenesis in *Thamnidium elegans* Link.
23. J. G. Manners, S. M. M. Hossain (Southampton). Conidial germination in *Erysiphe graminis*.
24. J. G. Manners, R. C. Jancey, W. T. Williams (Southampton). Some applications of statistical methods to fungal taxonomy.
25. W. M. Page (Birkbeck College). *Pleurage toenoides* in culture.
26. B. E. Plunkett (Birkbeck College). Tropisms and differentiation in *Polyporus brumalis*.
27. T. F. Preece (Plant Pathology Laboratory, Harpenden). A staining method for the study of apple scab infections.
28. J. E. Robson, H. M. Stockley (A.E.R.E., Harwell). Radio-active mercury compounds for the detection of sulphydryl groups in fungi.
29. J. Taggart, P. Swinbank, S. A. Hutchinson (Glasgow). Measurement of electrostatic charges on basidiospores.
30. J. Taggart, P. Swinbank, S. A. Hutchinson (Glasgow). The effect of tilting sporophores of *Polyporus betulinus* on the efficiency of spore liberation.
31. P. W. Talboys (East Malling). The use of 'Cellophane' sheets colonized by *Verticillium* spp. as inoculum in host-parasite studies.
32. H. T. Tribe (Cambridge). Sections of organic horizons of soils.
33. G. Valadon (Southampton). Heterothallism in *Protomyces inundatus* on *Apium nodiflorum*.
34. M. H. Zoberi (Birkbeck College). Take-off of mould spores.
35. D. M. Losel (Dublin), S. A. Hutchinson (Glasgow). The biological effects of volatile fungal metabolites.

# THE BRITISH MYCOLOGICAL SOCIETY

*Recognosce notum, ignotum inspice*

## RULES*

### *Society's Name and Objects*

1. The Society shall be called 'The British Mycological Society', its object shall be the study of Mycology in all its branches, and it shall publish the *Transactions of the British Mycological Society*.

### *Members of Society*

2. The Society shall consist of Honorary Members, Members (including Foundation Members†), and Associates; the number of Honorary Members shall be limited to 12 at any one time, but the number of Members and Associates shall be unlimited.

Members and Honorary Members are entitled to all the privileges of the Society; Associates may attend the Society's meetings and forays, but may not vote and are not entitled to receive the *Transactions* free of extra charge.

### *Honorary Members*

3. Honorary Members shall be persons of pre-eminence in Mycology or who have rendered special service to the Society.

### *Officers*

4. The Officers of the Society shall consist of a President, two or more Vice-Presidents, a Treasurer, a Secretary, a Foray Secretary, and the Editors of the *Transactions*.

### *Government of the Society*

5. The government of the Society shall be vested in a Council elected from among the Members, which shall consist of the President and other Officers, Past-Presidents who served before 31 December 1936, nine ordinary members of the Council and the Secretary of the Plant Pathology Committee *ex officio*.

The Council shall meet at least twice every year, eight members to form a quorum.

### *Election of Officers*

6. The Officers shall be elected annually at the Annual General Meeting of the Society by the following procedure. Nominations for each office shall be made by the Council and circulated to all Members at least

* See these *Transactions*, **43**, pp. 463 and 698.

† Foundation Members are those Members who joined the Society previous to the limit of 100 members having been attained. This was reached 22 October 1903.

two weeks before the Annual General Meeting. In making nominations the Council shall take into consideration recommendations made to it in writing by any Member, if received at least ten weeks before the Annual General Meeting. All nominations shall be voted upon at the Annual General Meeting.

The President, who shall serve for a term of one year, and the Vice-Presidents, who shall be the retiring President and one or more elected for a term of office of one year, shall not be eligible for immediate re-election. Other Officers shall be eligible for immediate re-election, but the Secretary and Foray Secretary shall not normally serve for an unbroken period exceeding five years.

*Election of Ordinary Members of the Council*

7. Ordinary members of the Council shall normally serve for three years, three members retiring each year. The members to retire, who shall not be eligible for immediate re-election, shall be those who have been longest in office, or where there is equality of service, those determined by the Council. Vacancies caused by such retiring members, or by other resignations (except under Rule 8) shall be filled by election by the following procedure. Each Member of the Society shall be invited to make nominations for ordinary membership of the Council, all such nominations to be duly proposed and seconded, and with the consent of nominees. The Council may also make nominations, with the consent of the nominees, and in doing so will take into account written recommendations made by any Member. Nominations and recommendations shall reach the Secretary not less than ten weeks before the Annual General Meeting. A list of all duly completed nominations shall be circulated to all Members at least two weeks before the Annual General Meeting, and the new ordinary members of the Council will be elected from this list at the Annual General Meeting, with a ballot at the meeting where the number of nominations exceeds the number of vacancies.

8. The Council may fill by co-option any casual vacancy which may occur among the Officers or ordinary members of the Council. Any person so co-opted shall serve only until the next Annual General Meeting. He shall be eligible for election to the Council at this meeting under Rule 7.

*Plant Pathology Committee*

9. The special interests of Plant Pathology shall be delegated to a Plant Pathology Committee. The Plant Pathology Committee shall consist of the President and Secretary of the Society, *ex-officio*, and twelve other Members of the Society. The latter shall be elected at the Annual General Meeting and each shall normally serve for four years, three retiring each year. The members to retire shall not be eligible for immediate re-election, and shall be those who have been longest in office, or, where there is equality of service, those determined by the Committee. Vacancies caused by such retiring members shall be filled by election at the Annual General Meeting of the Society, nominations being made by the Plant Pathology Committee

which shall take into consideration written recommendations submitted by any Member of the Society not less than ten weeks before the Annual General Meeting. Council shall circulate to all Members the list of nominations at least two weeks before the Annual General Meeting. Casual vacancies on the Committee may be filled by co-option, such co-opted members to serve only until the next Annual General Meeting. They shall be eligible for election to the Committee at this meeting.

The Officers of the Committee shall consist of a Chairman and a Secretary, elected from among its members by the Committee each year. The Secretary shall be an *ex-officio* member of the Council of the Society. He may hold office for a term not exceeding three years, irrespective of the length of his immediately preceding service as an ordinary member of the Committee.

The Committee shall meet at least twice each year, six members to form a quorum.

*Foray Committee*

10. The organization of the Society's forays and field work shall be delegated to the Foray Secretary, assisted by a Foray Committee of six other Members of the Society together with the President and the Secretary of the Society *ex-officio*.

The six ordinary members of the Committee shall be elected at the Annual General Meeting and shall normally serve for three years, two retiring each year. The members to retire, who shall not be eligible for immediate re-election, shall be those who have been longest in office, or, where there is equality of service, those determined by the Foray Committee. Vacancies caused by such retiring members shall be filled by election at the Annual General Meeting of the Society, nominations being made by the Foray Committee, which shall take into consideration any written recommendation received from a Member of the Society not less than ten weeks before the Annual General Meeting. The Council shall circulate to all Members the list of nominations made by the Foray Committee at least two weeks before the Annual General Meeting. Casual vacancies on the Committee may be filled by co-option, such co-opted members to serve only until the next Annual General Meeting, when they become eligible for election.

*Period of Office*

11. The Treasurer shall hold office from 1 July immediately following the Annual General Meeting. Other Officers, the ordinary members of Council, and members of all Committees shall hold office from 1 January immediately following the Annual General Meeting.

*Election of Honorary Members, Members and Associates*

12. Honorary Members shall be elected, on the recommendation of the Council, at a meeting of the Society by a majority of the Members present.

Every candidate for election as a Member or an Associate shall be proposed by two Members, who shall sign a certificate of recommendation, one at least of the proposers so recommending from personal knowledge.

All candidates shall sign an undertaking to abide by the Rules if elected. They shall be elected by a majority of the Members present at any meeting of the Society, or, subject to ratification at a subsequent meeting, by the President and other Officers of the Society.

*Subscriptions*

13. Each Member shall pay a minimum annual subscription of thirty shillings, each Associate ten shillings, and each Foundation Member five shillings, due on 1 January each year. Honorary Members shall be exempt from such payments.

Members of at least 25 years standing who are over 65 years of age (60 if already in retirement) may pay a subscription at half the minimum rate.

*Resignation*

14. A Member or Associate wishing to resign from the Society shall give notice in writing to the Secretary or to the Treasurer.

*Ordinary Meetings*

15. The Society shall hold meetings at places and times determined by the Members at the preceding Annual General Meeting, or by the Council.

*Annual General Meeting*

16. Each year, ordinarily in December, an Annual General Meeting shall be convened at which Officers, ordinary members of the Council, members of the Plant Pathology and Foray Committees and an Auditor shall be elected, audited accounts presented by the Treasurer, and any proposed alterations of the Rules voted upon as provided for in Rule 18. The Council shall send the Agenda for the meeting to each Member of the Society at least two weeks before the meeting. Thirty Members shall form a quorum.

*Special General Meetings*

17. Such meetings may be called by the Council, and shall be called by the Secretary on request in writing by not less than ten Members. The Agenda of the business to be transacted shall be sent to each Member at least two weeks in advance. Thirty Members shall form a quorum.

*Alteration and Circulation of the Rules*

18. Proposals for alteration of the Rules may be made by the Council or by not less than ten Members. Adoption of such changes shall be subject to a two-thirds majority of those Members present at a Special General Meeting, and ratification by a like majority at an Annual or Special General Meeting held not less than two months later. A copy of such proposals shall be sent to each Member with the Agenda for each meeting.

A printed copy of the Rules shall be sent to each Member on election, and, in the event of any major alteration, to all Members.

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